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ENVIRONMENTAL INFLUENCES ON NECTAR SECRETION

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This study was undertaken to summarize and supplement existing knowledge of the factors which stimulate or retard the secretion of nectar. The work was carried out under the direction of the botanical section of the Iowa State Experiment Station in cooperation with the chemical section, being done mostly at Ames, Iowa, from June 1914 to June 1916.

Historical

One of the most complete treatises on nectar, with quite an extensive account of some of the environmental factors in its secretion, was written by BONNIER (1). The subject of secretion has been much debated from a physical standpoint. GODLEWSKI (9) attributes it to a fluctuation in the concentration of the cell sap due to alternate splitting and recombination of complex molecules. PFEFFER (19) advances 3 possible causes for secretion: (1) an unequal permeability of the membrane of the absorbing and excreting portions of the cell; (2) an unequal distribution of solutes in the absorbing and excreting portions of the cell; (3) the transformation into sugar of the outer portion of the cell wall, and the osmotic action of this sugar upon the liquid contents of the cell. LEPESCHKIN (14), in a study of the coenocytic plant *Pilobolus*, finds

evidence that the first of PFEFFER's theories is the correct one for the excretion of water drops. WILSON (**29**) gives evidence in support of PFEFFER's third theory, showing that the thorough washing of a nectary stops the secretion if the nectary is past the stage of metamorphosis of the cell wall, but that secretion is resumed on the addition of sugar to the surface of the nectary. The validity of his results is called in question by LEPESCHKIN (**14**) and BÜSGEN (**5**). HAUPT (**10**) in a study of extrafloral nectaries finds that some nectaries become inactive after washing, while others, as those of the leaves of *Impatiens parviflora*, continue excretion of water but not sugar, thus becoming equivalent to hydathodes. LIVINGSTON (**16**) likens nectar secretion to guttation, accounting for the latter by a decrease in the permeability of the plasma membrane induced by an increased turgidity, and for the former by a hypothetical rapid increase in the solute content and thereby of the osmotic pressure in the cell, a change which induces a like decrease in the permeability of the membrane.

Comparatively little work has been done on the chemistry of nectar. WILSON (**28**), VON PLANTA (**27**), and BONNIER (**1**) have analyzed a few kinds of nectar, finding that in some cases it contains no sucrose, while in others it is almost wholly this kind of sugar. In some cases fructose and in others glucose is the dominating reducing sugar. The sucrose of nectar is almost wholly digested in honey, BROWNE (**4**) finding as the average composition of 138 honey samples from widely separated localities 38.65 per cent fructose, 34.48 per cent glucose, and 1.76 per cent sucrose.

Investigation

METHODS

Nectar when secreted in sufficient quantities was measured by means of a graduated capillary pipette, or weighed after absorption on strips of filter paper which had previously been weighed in small vials. Many of the most important honey plants secrete such small amounts of nectar to the individual flower that neither of these methods is practicable. In these the amount of sugar external to the nectaries was determined approximately by adding a definite

volume of water to a counted or weighed quantity of the flowers, shaking frequently for half an hour, then decanting. A similar method was employed by VON PLANTA (**27**) and BONNIER (**2**). In some of the flowers investigated, this treatment extracts some sugar from the floral tissues, as shown by the appearance in the solution of colors from the floral envelopes, hence it is of value mainly for the comparison of flowers of the same species. Buckwheat, because of its rapid maturing, its value as a honey-producing crop, as well as its comparative freedom from this source of error, was employed in many of the experiments.

Sugar determinations were made by reduction of Fehling's solution. The method found most practicable and employed for the greater part of the work was based on that described by SCHOORL (**24**). A carefully measured amount (1 cc. for minute quantities of sugar, 10 cc. of the material to be analyzed in a 150 cc. Erlenmeyer flask) was heated on an asbestos gauze over a flame so adjusted that the liquid began to boil in just 2 minutes, and then was boiled for 2 minutes longer. To the contents of the flask after cooling to 60° C. were added sulphuric acid and potassium iodide. The liberated iodine, which corresponds to the unused copper sulphate, was titrated against sodium thiosulphate. Sugar values were obtained by the careful analysis of known quantities of sugar. This method has the advantage of being both rapid and delicate enough to determine minute quantities of sugar with a probable error of not over 0.04 mg. Floral tissues, when not too bulky, could be analyzed by the same method, the reagents being added directly to the tissues after covering them with water. When tissues were more bulky or when greater accuracy was required, extractions were made with alcohol or water, and were purified by treatment with neutral lead acetate.

HUMIDITY

It is a well known fact that any watery exudation from plants accumulates when atmospheric humidity is high and evaporation is thereby retarded. This can easily be demonstrated in connection with bleeding from severed tissues or with guttation through water stomata. BONNIER (**1**) states that nectar secretion corresponds to guttation and that it varies inversely with the transpiration.

So far as the volume of nectar is concerned, I have found this to be true in all the plants experimented upon with this end in view. But there are two factors involved in nectar secretion, as shown by PFEFFER (**20**), the exudation of water and that of sugar. HAUPT (**10**) has found that extrafloral nectaries begin secreting only when humidity is relatively high, an observation which confirms the theory that secretion is due to a decreased permeability caused by increased turgor, but that after secretion begins increased air moisture increases water secretion, the secretion of sugar remaining constant. It is probable that this applies to nectaries in general. Nectar is more dilute when humidity is high, and honey that is stored at such times is likely to be high in water content.

At Ames the seasons of 1914 and 1915 represented extremes of humidity, the summer months of the former year being excessively dry and warm, while those of the latter year were excessively wet and cool. Hence comparisons of nectar washed from flowers, as given in table I, are of interest.

TABLE I

Species	1914		1915	
	Number of samples analyzed	Average mg. sugar per gm.	Number of samples analyzed	Average mg. sugar per gm.
Melilotus alba, flowers	6	2.13	3	0.65
Medicago sativa, flowers	4	1.15	3	0.80
Trifolium pratense, corollas	4	3.64	13	3.00

It is seen that the wet season yields rather less sugar than the dry. It may be stated further that bee visitors to *Melilotus* were several times as abundant in 1914 as in 1915. I have found by experiment that flowers of alfalfa grown in dry soil contain about 60 per cent more sugar than those grown in wet soil.

Buckwheat flowers kept humid under a bell jar secreted much more liquid than flowers exposed to the rather dry greenhouse air. However, 12 comparative analyses of the nectar of each show 1.04 mg. sugar per 100 blossoms in the humid, and 0.98 mg. sugar per 10 blossoms in the dry. Analysis of the flower after removal of the nectar in 6 of the above pairs of cases shows 0.74 mg. per

10 blossoms in the humid and 0.98 mg. per 10 blossoms in the dry. More sugar accumulates in a dry atmosphere and practically the same amount is excreted.

The accumulation of sugar under low moisture conditions is in line with the discovery by LUNDEGARDH (**17**) that increase of moisture favors the accumulation of starch; decrease of moisture favors its digestion.

Six plants of *Impatiens sultana* in saturated air accumulate in a day 3.26 mg. sugar each from the extrafloral nectaries (the basal teeth of the leaves), while 6 plants in greenhouse air accumulate 5.42 mg. sugar each. The excess of the latter is very likely due in part to the running away of drops under the humid conditions. The nectar averages 23.4 per cent sugar in the former and 45.3 per cent in the latter.

RAINFALL

The author has shown in a statistical study (**12**) that heavy rainfall just before the secreting season is advantageous, as it gives the plants greater vigor. But during the season of greatest secretion good years are somewhat drier than poor. Also a rainy day shows a lighter honey yield than a day before or after the rain. The deterrent effect of the rain on the honey flow is twofold: it hinders the activities of bees and it washes away the nectar. To illustrate the latter point, in 1915 on the morning following a day of continual rainfall, red clover corollas were found to contain 0.02 mg. sugar per gm., whereas a day earlier they contained 3.8 mg., a day later 0.6 mg., and 2 days later 4.4 mg. Buckwheat blossoms were subjected to an experiment to determine the extent to which rains wash away the nectar. Flowers subjected before gathering to a spray for 20 minutes, 15 mm. of water falling, were found to contain 0.12 mg. per 10 as against 1.28 mg. per 10 of untreated flowers. A 30-minute rain of 35 mm. reduced the nectar of red clover blossoms from 0.48 to 0.19 mg. per 10, and that of white clover blossoms from 0.27 to 0.07 mg. per 10.

TEMPERATURE

WILSON (**29**) states that temperature has not a marked effect upon the rate of secretion of nectaries that have commenced

secreting. He finds, however, that *Prunus laurocerasus* will not begin secretion unless the temperature is 12° C. or over. HAUPT (10) also finds that a minimum temperature is necessary to induce secretion. LEPESCHKIN (14) finds in the hyphae of *Pilobolus* a secretion steadily increasing with, and much more rapidly than, the absolute temperature. In other cases he finds an optimum above which secretion diminishes. In the case of secreting hairs of the bean leaf this optimum is 20°, in the *Abutilon* nectary it is 26°.

Experiments were carried out in uniform temperature incubators. For much of the work, to avoid light exclusion, which is detrimental to secretion, incubators were employed which were specially constructed for the purpose, being covered with two glass plates separated by an air space.

The optimum temperature for amount of secretion lies between 20° and 25° for *Cucurbita Pepo*, *Lilium speciosum*, *Canna indica*, *Euphorbia pulcherrima*, and extrafloral nectaries of *Impatiens Sultani*. For *Salvia splendens* and most of the Leguminosae tested it is about 15°. As a rule, the sugar concentration of the nectar does not differ materially for the different temperatures. Typical sugar determinations obtained from the flower of *Abutilon striatum* are given in table II, the blossoms being quartered and one piece of each placed in each incubator, thereby eliminating any error due to individual variations.

TABLE II

Time	Mg. invert sugar per flower			
	10°	16°	23°	30°
After 36 hours	10.20	12.00	16.00	10.32
After 16 hours (another set)	3.07	6.57	12.97	10.87

Here the optimum is clearly not far from 23°.

BONNIER and FLAHAULT (1, 3) call attention to the fact that nectar secretion is greater in the same species at high latitudes and altitudes than at low when the species grows normally in both latitudes or altitudes compared, and furthermore that species which do not secrete in France are nectariferous in Norway and in the Alps. He suggests that this fact may be due to the greater range

between maximum and minimum daily temperatures which prevails at high altitudes and latitudes, or to the greater range in the humidity of the air.

PHILLIPS (**21**) observes that alfalfa in general is valuable as a honey plant in the Great Plains region of the west and not in the eastern states; that buckwheat is of more value in New York, Pennsylvania, and Michigan, than in Indiana and Illinois; and that white clover is of greater importance in the north than in the south. Basswood is said to secrete better in the more northerly portions of its range. It seemed desirable, therefore, to investigate the hypothesis of BONNIER.

As I have shown (**12**), the study of a 30-year weight record of a hive at Clarinda, Iowa, lends strong support to this assumption. Thirty-eight periods of continual and fairly rapid gain in weight were selected, and the days of each divided about equally between days of high gain and days of low gain. In 32 cases the average diurnal temperature range for the days of high gain was greater than that for the days of low gain. In all of the 6 exceptional cases the difference between the average was small. SLADEN (**25**) states that the heaviest single day's increase in hive weight noted for two seasons in England in a record kept by EDE was on a day that began with a heavy early morning frost, the honey coming from the heather (*Calluna vulgaris*).

Table III represents the amount of reducing sugar in mg. which the author found after keeping the plants or flowering branches for a time in the incubators.

In field conditions it can readily be shown that lower temperatures increase the sugar content in dandelion and the clovers.

How does high temperature influence secretion? VAN RYSSELBERGHE (**26**) determined that with increase in temperature the permeability of the protoplast to water and solutes rapidly increases, that of *Tradescantia* epidermal cells for water being 8 times as great at 30° as at 0°, and that for solutes seeming to follow the same proportional rule. To demonstrate whether this holds for nectary cells, I determined the lowest sucrose concentration necessary to plasmolyze the multicellular secreting hairs which cover the nectary of *Abutilon*. After 4 days at 10° a 0.6 molecular solution is

sufficient; while for another portion of the same flower, after 4 days at 25° a 1.1 molecular solution is necessary.

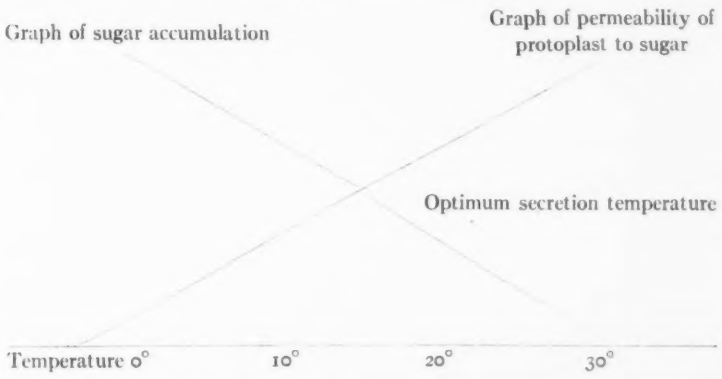
TABLE III

Species	10°	19°	25°	30°	10° (23° over night)
Trifolium incarnatum, 10 corollas, 2 days	0.56	0.63	0.43	0.38	.....
Trifolium repens, 10 flowers, 2 days	.....	1.12	0.04	0.70	.....
Nectar washed from same	.....	0.07	0.02	0.01	.....
Trifolium repens, 10 flowers, 2 days	0.93	0.63	0.47	.....	0.56
Nectar washed from same	0.0	0.0	0.0	.....	0.08
Medicago sativa, 10 flowers, 4 days	3.44	2.08	0.52	.....	.....
Caragana frutescens, 10 flowers, 2 days	37.55	22.22	11.95	.....	24.33
Nectar washed from same	0.64	4.80	2.00	.....	2.88
Fagopyrum esculentum, 10 flowers, 4 days	0.69	0.73	0.58	.....	.....
Nectar washed from same	1.36	0.80	0.0	.....	.....
Salvia splendens, 10 corollas, 4 days	.....	25.7	13.8	12.9	.....
Coleus Blumei, 10 corollas, 4 days	.....	0.86	0.78	0.62	.....
Taraxacum officinale, per gm. flowers, 1 day	38.7	45.0	19.3	13.9	.....
Taraxacum officinale, per gm. flowers, 1 day	15.6	17.1	11.9	not developing	.....

What is the influence of low temperature? MÜLLER-THURGAU (18) finds that sugar accumulates in potatoes when the surrounding temperature is below 10° C., and the same is true of hemp seedlings and various other plant organs. He advances the theory that the accumulation comes from the digestion of starch or oil more rapidly than the resulting sugar can be utilized, its respiratory destruction being retarded by the low temperature.

The accumulation of stored sugar from starch in low temperature in the twigs of woody plants is a well known phenomenon which is amply discussed by FISCHER (8). Indeed this accumulation seems to be rather common in its occurrence among plant tissues. Besides being applicable to floral tissues, as table III clearly shows, it affects the leaves and the peduncles of white clover, the former after 2 days' treatment having 30 per cent more sugar and the latter 58 per cent more at 10° than at 25°. The evidence points to the conclusion that at a uniform temperature the secretion

of nectar is a balance between two factors, namely, the accumulation of sugar in and near the flower under the influence of low temperature, and increasing permeability of the plasma membrane under the influence of high temperature. The position of the optimum, then, might be represented somewhat as follows:


Graph of sugar accumulation
Graph of permeability of protoplast to sugar
Optimum secretion temperature
Temperature 0° 10° 20° 30°

The two graphs are limiting factors to nectar secretion, and the intersection, that is, the point where the effective limit stands highest, is the optimum secretion temperature. If the fact discovered by ECKERSON (7) for root cells, that above a certain point (25–35°) the permeability again decreases, applies also to nectary cells, the situation may be somewhat complicated thereby.

Better than any uniform temperature for secretion is a change from a lower to a higher temperature, as table III indicates. The influence of such a change might be graphically indicated by folding the above diagram so that two temperatures, say 10° and 30° are brought together. Both limiting factors are raised; the sugar which has accumulated at the lower temperature is secreted at the higher.

ATMOSPHERIC PRESSURES

In the study previously cited (12), I have shown that of 18 periods of continual honey production, 16 have a lower barometric pressure on the days of heavier yield than on the days of lighter yield, the two exceptional cases having very slight differences. The increased secretion already credited to high altitudes might be

attributed to the diminished pressure, but this explanation would not account of course for the similar increase at high latitudes.

In investigating experimentally the influence of pressure, I covered the plant under experiment with a tabulated bell jar waxed to fit tightly to a ground glass plate and connected by means of a stopcock with the water aspirator. A similar plant was placed under a control bell jar. In some of the experiments air was daily renewed in both low pressure and control jar; in others its continual renewal was provided for by admitting a current of air which bubbled through water, and in the case of the low pressure jar entered by means of a capillary tube with a very small aperture. This latter method is similar to one employed by SCHAIBLE (**22**). By the use of an aneroid barometer the pressure was maintained at about 50 cm., or two-thirds atmospheric pressure, the prevailing condition at altitudes of about 10,000 ft. Repeated investigations were made with the following plants: for guttation, *Tropaeolum majus* and *Avena sativa;* for nectar secretion, *Tropaeolum majus*, *Impatiens Sultani*, *Abutilon striatum*, *Euphorbia pulcherrima*, *Canna indica*, *Fagopyrum esculentum*, *Salvia splendens*, *Coleus Blumei*, *Antirrhinum majus*, and *Prunus americana*.

There were no constant differences in secretion which could be detected by either physical or chemical means. It is very doubtful, therefore, whether the much smaller variations in pressure which occur in nature could measurably affect nectar secretion. It is a matter of common knowledge among beekeepers that bees are more active when the barometer is low, the warmth and stillness of such periods favoring activity. Hence it seems very probable that any relation between atmospheric pressure and honey flow is to be attributed to the bees and not to the plants.

LIGHT

DARWIN (**6**), WILSON (**29**), and HAUPT (**10**) note the fact that the extrafloral nectaries of several species of *Vicia* are stimulated to activity by light. The first author adds *Lobelia erinus* and the last the Euphorbiaceae as plants that require the light stimulus for secretion. The two latter authors, however, state that in the greater majority of cases secretion is only indirectly related to

light. HAUPT finds that in most extrafloral nectaries even disturbances in photosynthesis by darkness show their influence on secretion only very slowly. Light in *Vicia* doubtless increases the permeability of the protoplast, as LEPESCHKIN (**15**) has found that it does in the pulvini of Leguminosae in general.

SCHIMPER (**23**) found that extrafloral nectaries on the leaves of *Cassia neglecta* cease their activity in a few days when the plant is kept in darkness or in an atmosphere deprived of carbon dioxide, but that secretion continues when the leaf is in the light and only the nectaries are darkened.

I experimented upon both the floral and the extrafloral nectaries of *Impatiens Sultani*, and it seems clear that the withdrawal of light makes its influence fairly rapidly and very decidedly felt. Table IV gives a typical study of floral nectaries, the measurements being millimeters in length of the part of the spur which contains nectar. The table includes average increases over last measurement of the spur in those flowers which were open when the last record was taken, and the average measurement for those flowers which have opened since the last record. One plant was covered by a bell jar, the other was covered by an opaque jar of about the same size.

TABLE IV

Days	Light		Dark	
	Gains	New flowers	Gains	New flowers
2	4.4	21.6	1.2	16.1
3	4.8	22.0	1.1	13.6
4	2.0	23.0	1.1	15.7
6	3.5	29.0	−1.2	17.3
7	5.0	20.0	0.1	16.0
8	4.1	21.7	0.5	14.0
9	3.3	19.0	−0.4	13.0
10	3.2	22.0	−0.5	0.0

At this time the dark plant had taken on an etiolated appearance and new flowers were scarcely developing. Secretion from extrafloral nectaries had practically stopped after 3 days in the dark.

Half the leaves of a plant were covered with black tissue paper which was fastened by means of small brass paper clips, only the

basal or nectar-secreting teeth being left uncovered. These leaves secreted very little after the third day, whereas the uncovered leaves of the same plant were uninterrupted in their secretion.

Buckwheat flowers were gathered at the same time from under light and dark jars, and it was found that after two days in the dark, although the total amount of liquid secretion was not in the least diminished, the proportion of sugar began to decrease, the secretion not tasting sweet or giving a very positive sugar test. An average of 11 such analyses of the nectar of flowers that had been covered for 2–8 days gives per 10 blossoms 1.20 mg. invert sugar in the light, and 0.41 mg. in the dark. Sugar contained in the flowers does not differ greatly, however, in the two cases, there being 0.79 mg. to 10 flowers from the light and 0.73 mg. to 10 flowers from the dark. Plants which had been left in the dark for some time continued to secrete less than normal quantities of nectar for a week or more after the removal of the cover.

That the diminution of sugar is due to the interference with photosynthesis may be shown by removing all the leaf blades from a number of plants. Eleven analyses average in milligrams invert sugar in the nectar of 10 flowers 0.36 from plants with leaves removed 3–10 days, and 0.85 from the normal plants serving as checks. Seven of the above pairs of cases give for the entire invert sugar content of the flowers 0.59 mg. from the mutilated and 0.92 mg. from the check.

The same result may be gained by covering all the leaves of the plant with black tissue paper, and comparing the nectar with that of normal plants. Here we find as an average of 6 analyses of 10 flowers 0.23 and 0.69 mg. invert sugar respectively in the nectar; 0.83 and 1.40 in the whole flowers. After 4 days in the dark there is approximately one-fourth as much sugar secreted per flower.

When the flowers only are covered from the light, they secrete fully as much sugar as those not covered; so the extrusion of sugar is clearly dependent upon the food reserves of the plant.

I found the same relation to exist in the *Canna* blossom. Darkening of the entire plant materially diminished the nectar secretion, while darkening the flower cluster alone had no influence upon it.

Other flowers analyzed, among them being *Antirrhinum majus*, *Cucumis sativus*, *Salvia splendens*, and *Coleus Blumei*, contain less sugar and secrete less sugar when kept in the dark; furthermore, the nectar is usually less in volume. *Euphorbia pulcherrima* does not begin secretion in a dark chamber, and *Abutilon striatum* secretes only very slowly and very scantily. Even plum branches, which contain supplies of stored food, when developed in the dark have little more than half as much sugar in the tissues and about one-fourth as much nectar as when developed in the light.

FERTILITY OF SOIL AND VIGOR OF PLANT

HUNTER (11) states that alfalfa yields the greatest amount of nectar under conditions that tend to give it the most vigorous growth, proper heat, and moisture upon suitable soil. All of my observations and experiments tend to confirm this for the various plants investigated. Red clovers grown on fertilized plots were found to contain slightly more sugar and to secrete slightly more as nectar than those on the unfertilized control plots adjoining.

BONNIER (2) has experimented on the secretion of several plants as influenced by different soils. He finds that *Sinapis alba*, *Isatis tinctoria*, and *Medicago sativa* yield most nectar on limy soil, while *Phacelia tanacetifolia* does better on clay and *Fagopyrum esculentum* on sand. It is probable that soils which are conducive to greater vigor and more surplus food in the plant are on the whole more favorable for nectar yield.

White clover, the leading honey plant of this section, collected the same day in the same part of the city gives the tests shown in table V.

TABLE V

CONDITION	SUGAR PER GM. OF FLOWERS IN	
	Nectar	Flowers
Stunted by rank growth of weeds	0.2	15.9
Stunted by close mowing	0.6	20.8
Vigorous	3.7	21.6

Vigorous plants of buckwheat yield about twice as much nectar as do weak ones in the same bed. The flowers are found on analysis

to contain 20–50 per cent more sugar. Plants which had been allowed to dry to the wilting point several times in the course of their growth and were consequently stunted to about one-half normal height yielded less nectar, the average of 6 comparisons being 0.44 mg. per 10 flowers of the stunted and 1.39 mg. per 10 flowers of the normal. Plants grown in a greenhouse in which the temperature was low and which consequently were stunted to about one-third normal height, not blooming until twice the age of normal blooming plants, secreted practically no nectar.

It was noticed further that a *Salvia* deeply rooted in the soil secreted more than one which was hampered by a small pot, and that of the former plant young vigorous branches yielded more nectar than old stunted ones.

PORTION OF FLOWERING PERIOD AND AGE OF FLOWER

Table VI illustrates the relation of nectar secretion to the part of the flowering season in which the flower in question appears. In all cases the compared flowers were collected on the same day, but from patches varying in stage development.

TABLE VI

Species	Early in blooming season		Late in blooming season	
	Sugar in nectar	Sugar in flowers	Sugar in nectar	Sugar in flowers
Trifolium pratense	9.3	20.7	4.6	18.6
Medicago sativa per gm. of flowers	1.5	38.9	0.3	26.8
Buckwheat 10 flowers (average of 4 tests)	1.62	0.72	1.09	0.60

As a rule, the younger plant is undergoing more active photosynthesis and has greater reserves of food to be secreted by the nectaries.

Kurr (13) makes the statement that secretion of nectar commences very rarely before the dehiscence of the anthers; it is generally most rapid during the pollination period; and it ceases as soon as the fruit begins to develop. Bonnier (1) agrees to this proposition and asserts that nectar is simply a manifestation of the surplus of food stored in the nectariferous part corresponding to an

arrest in the development of the organ. In the case of floral nectaries, therefore, it is most pronounced after the ovary has attained maximum development and before the fruit has commenced to develop. He finds a maximum proportion of sucrose in the floral organs corresponding to this time of greatest secretion. Chemical analyses of the floral tissues show that the climax of sugar accumulation is about the time of the dehiscence of the stamens, and that as the flower withers there is a very rapid decrease in the amount of sugar. Table VII gives some examples from my work, the tissues having been extracted and purified.

TABLE VII

Species	Buds		Mature flowers		Declining flowers	
	Invert sugar	Sucrose	Invert sugar	Sucrose	Invert sugar	Sucrose
Medicago sativa per gm. . .	32.5	2.8	65.4	0.0	26.1	0.0
Melilotus alba per gm.	22.2	2.6	31.1	Trace	. . . . .	. . . . .
Melilotus officinalis per gm.	16.0	0.0	28.8	0.0	. . . . .	. . . . .
Trifolium repens per gm. . .	8.1	0.1	8.3	1.8	7.5	0.0
Trifolium hybridum per gm.	13.8*	. . . . .	8.6*	. . . . .	. . . . .	. . . . .
Taraxacum officinale per gm.	17.8	. . . . .	27.2	. . . . .	. . . . .	. . . . .
Impatiens Sultani per 100 flowers	47.5	0.0	248.4	0.0	190.0	48.0
Lilium speciosum rubrum per flower.	119.5	26.7	179.3	10.9	76.0	1.0
Lilium longiflorum per flower. . . . . younger	51.4	14.9	95.2	11.3	22.2	0.0
Lilium longiflorum per flower. . . . . older	95.9	21.3				

* Including sucrose.

It seems that in most cases the excess of sucrose, the storage form of sugar, is rather before the flower opens and nectar secretion begins.

Summary

1. By increasing humidity the secretion from nectaries of water but not that of sugar is increased.

2. Excessive water supply lessens the sugar surplus in the parts of the flower.

3. Dilution and washing by rain cause much of the sugar of nectar to be lost.

4. Rate of secretion for both sugar and water increases with temperature up to a certain optimum.

5. Accumulation of sugar in the flower and its vicinity varies inversely as the temperature.

6. The optimum condition for sugar secretion is an alternation of low and high temperatures.

7. Variation of atmospheric pressure has no marked influence on secretion.

8. Sugar excretion is markedly diminished in darkness on account of limitation of the food reserves of the plant. Water excretion may or may not continue, depending upon the species. Removal of the leaves has the same deterrent effect.

9. The more favorable all conditions for growth and the more vigorous the plant, the greater is the amount of sugar secreted.

10. Nectar is most abundant early in the blooming season, other things being equal.

11. Accumulation and secretion of sugar is most pronounced near the time of the opening of the flower.

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DEVELOPMENT OF EMBRYO SAC AND EMBRYO IN EUPHORBIA PRESLII AND E. SPLENDENS

WANDA WENIGER

(WITH PLATES XIV–XVI)

Introduction

LINNAEUS had considered the cyathium of *Euphorbia* to be a single flower, its involucre a perianth, and the staminate flowers stamens. The inaccuracy of these assumptions was demonstrated by BROWN (**2**), who substituted for them the conceptions still held, namely, that the cyathium consists of an involucre containing a single pistillate flower and many staminate flowers.

The earliest monograph of the Euphorbiaceae was that of BAILLON (**1**), in 1858. He found that a character common to all species of the family is the uniovulate or biovulate locule. The anatropous or orthotropous ovule becomes a seed with 3 seed coats, the outer one of which usually disintegrates. The embryo is surrounded by an oily endosperm and has a rudimentary root cap. BAILLON'S figures, reproduced by STRASBURGER (**20**), show the structure of the nucellus and embryo of *E. Lathyrus*. The name "obturateur" was applied by BAILLON to the mass of cells which grows from the placenta, pushing into the micropyle, and which is thought to determine the direction of growth of, and to nourish, the pollen tube. The nucellus grows out into a beak before the time of fertilization and the cells of the obturator grow close to the nucellus.

The obturator was called by MIRBEL (**9**) a "chapeau de tissu conducteur," by PAYER (**15**) a "capuchon," and by CAPUS (**3**), who studied *E. Myrsinites* in particular, a "coussinet micropylaire." POISSON (**16**) describes the integuments and obturator of *E. Lathyrus* and *E. Peplis*. PAX'S account (**14**) of the structure of the ovule agrees with that of BAILLON. According to him, however, caruncula and obturator are one and the same structure.

Miss LYON (**8**) gives a full account of the life history of *E. corollata*. There are 3 carpels in each pistillate flower, forming a tri-

locular ovary with a single suspended anatropous ovule in each locule. The inner integument appears first, but it remains small, while the outer integument grows beyond it. The megaspore mother cell is subepidermal in origin, but the cells of the nucellus, and possibly those of the epidermis near the upper end of the embryo sac, divide with great rapidity, producing a long, slender neck, and leaving the embryo sac deeply imbedded. The embryo sac develops in the usual way from the lowest of a row of 4 axial megaspores. The synergids are extremely long and the egg is situated between them. The pollen tube passes between the synergids, and the fusion of the male nucleus with the egg nucleus was observed. The fusion of the polar nuclei takes place near the egg. The antipodal cells are ephemeral and were seen by Miss LYON but once. The neck of the nucellus and the "glandular hairs," as the cells of the obturator are called, disintegrate after the entrance of the pollen tube, and the outer integument closes the mouth of the micropyle.

HEGELMAIER (**6**) reports habitual polyembryony in *E. dulcis*. From 2 to 9 embryos appear at the micropylar end of the sac. One embryo, which comes from the egg and may be distinguished from the others by the presence of a suspensor, develops into the single embryo of the seed. Some of the supernumerary embryos come from the nucellus. Two of them often reach the cotyledon stage, with tissue systems differentiated; the other embryos appear as irregular masses. Since the cyathium of this species has a very small neck, HEGELMAIER thinks it improbable that the flowers are insect pollinated. Wind pollination is also improbable, because of the regularity with which seeds are formed in the locules. In a later paper HEGELMAIER (**7**) admits that, although fertilization in *E. dulcis* was not observed and although its possibility seems lessened by the sterility of a large proportion of the pollen grains, he cannot prove that it does not occur. Fertilization is not necessary for the production of the embryos from nucellar cells. There is a possibility that apogamy and also parthenogenesis occur.

ROEPER (**17**) reports observing 2 embryos in the seed of *E. platyphylla*. According to DE CANDOLLE (**5**), 2 embryos are also formed in *E. helioscopia*. SCHWEIGER (**19**) describes the obturator, nucellus,

and caruncula of many species of *Euphorbia*. The outer integument always develops before the inner. The obturator of *E. Myrsinites* begins as a small outgrowth from the placenta when the outer integument has grown almost half-way to the tip of the nucellus. The cells of the obturator increase rapidly, the outer ones becoming long and hairlike. At the time of fertilization, the obturator is mature and has the shape of a bell which fits over the micropyle. The long cells of this structure completely fill the space between the nucellus and the inner and outer integuments, but never grow into the nucellar tissue. The obturator gradually disintegrates as the embryo enlarges, until at the maturity of the seed it is represented only by a small swelling on the placenta. The form and size of the obturator differ in different species. The nucellus has a long, slender tip which is surrounded by the cells of the obturator. The caruncula is formed from the outer integument, after the embryo has developed. A row of cells differentiates it from the seed proper. This structure resembles a cap and aids in loosening the seed from the placenta at the time of dispersal. SCHMIDT (**18**) finds more than one megaspore mother cell in *E. palustris*. These are situated deep in the cells of the nucellus. His account of the development of the flower agrees with that of Miss LYON (**8**).

MODILEWSKI (**11**) describes an unusual development of the embryo sac in *E. procera*. The first 4 nuclei of the embryo sac are arranged in the form of a cross. Two divisions result in the formation of 4 tetrads of nuclei. One nucleus from each group migrates to the center of the sac, where the 4 unite. The mature embryo sac contains an egg apparatus, 3 antipodal cells, and 2 groups of 3 nuclei each, lying on opposite sides of the sac. In fertilization, one male nucleus fuses with the egg nucleus, and the other male nucleus fuses with the quadrivalent fusion nucleus in the center. The synergids, antipodals, and nuclear groups at the sides of the sac disintegrate. No case of polyembryony was observed. Later, MODILEWSKI (**12**) described the events preceding embryo sac development in *E. procera*. An archesporial row of 3 or 4 cells was found, each ultimately containing 4 nuclei. Only one of the 4-nucleate cells develops into an embryo sac. Often one or more

of the other cells of the row adheres to the developing sac for some time before it disintegrates.

According to MODILEWSKI, the embryo sacs of *E. Lathyrus*, *E. salicifolia*, *E. globosa*, *E. meloformis*, *E. Cyparissias*, *E. coralloides*, *E. variegata*, *E. helioscopia*, *E. Gerardiana*, *E. Ipecacuanhae*, and *E. heterophylla* develop normally. DESSIATOFF (4) describes the formation of 16 nuclei in *E. virgata*, in a manner similar to that described by MODILEWSKI for *E. procera* (11). In a still later study, MODILEWSKI (13) finds 16 nuclei in the embryo sac of *E. palustris*. The development proceeds exactly as in *E. procera*. On the other hand, the embryo sacs of *E. virgata* and *E. lucida* develop in the ordinary way. MODILEWSKI, whose material for the study of *E. virgata* was collected from various localities, disagrees with DESSIATOFF's notion of the structure of the embryo sac in this species (4). He thinks that the nuclei in DESSIATOFF's figures of the embryo sac resemble endosperm nuclei more than they do those of ordinary embryo sacs. He finds, also, that at the 2- and 4-nucleate stages the megaspore enlarges so rapidly that its wall becomes indistinct, and that the cells of the nucellus, which have been pushed aside in the growth of the spore, might easily be taken for nuclei of the developing gametophyte. DESSIATOFF, according to MODILEWSKI, mistook either endosperm or nucellar nuclei for nuclei of the mature embryo sac. If this is not the explanation of his results, MODILEWSKI thinks DESSIATOFF was mistaken in the determination of the species studied. MÖBIUS (10) has recently figured the relation of the integuments and obturator to the nucellus in *E. macrorrhiza*.

E. procera and *E. palustris*, on the present evidence, seem to be the only species of *Euphorbia* studied which deviate from the usual history of the embryo sac. In these species, MODILEWSKI found that since the endosperm nuclei are very large and usually contain 2 or 3 nucleoles, there is no danger of their being confused with the nuclei of the mature embryo sac.

Material and methods

Flowers and seeds of *Euphorbia Preslii* were collected in different stages of development during July and August 1915, along

railroad tracks in Madison, Wisconsin. They were fixed in Flemming's strong, medium, and weak fixatives, the first named giving the best results. Young buds, flowers, and seeds of *E. splendens* were fixed in various fixing solutions, including Flemming's, Carnoy's, and Juel's, and acetic alcohol fixatives. The best results in this case were obtained with the latter, the Flemming solutions failing to penetrate soon enough, due to the great amount of latex in all portions of the plants. The material was obtained during March and April 1916, from plants grown in the greenhouse. Sections were cut 5 or 6 μ in thickness. Some sections of embryos 10 μ thick were made. Flemming's triple stain was used with good results.

Observations

Euphorbia Preslii

Cyathium

The first evidence of the formation of the cyathium in this species is the appearance of a papilla (fig. 1, *p*) between 2 bracts (*b*) at the end of a peduncle. At the base of this papilla, staminate flowers (fig. 2, s^1) and the bracts of the involucre (*in*) soon appear. The outer bracts (*b*) continue to grow up about the developing cyathium. Ovules (fig. 3, *o*) next arise as small swellings on the central papilla (*p*). The carpels (*c*) of the pistillate flower appear at the base of the papilla and gradually grow up about it, forming a short style and 3 deeply 2-lobed stigmas (fig. 4, *sg*). The involucre now grows up about the staminate and pistillate flowers. The staminate flowers never extend above the neck of the involucre; in the early stages of the history of the pistillate flower only the stigmas project beyond the neck of the involucre. The pistillate flower consists of a single pistil, whose trilocular ovary terminates a stalklike structure which is jointed below to the pedicel (fig. 32). Soon after fertilization the stalk of the pistillate flower elongates, causing the pistil to project from the cyathium and nearly to close the opening of the involucre. When a stamen is nearly mature a depression appears, marking the point of juncture of the pedicel and the filament. Secondary staminate flowers arise as branches from the older ones (fig. 3, s^2). This description of the develop-

ment of the cyathium agrees with that given by Miss LYON (**8**) for *E. corollata*.

Embryo sac

A single ovule (fig. 4, *o*), which soon becomes anatropous, is formed in each locule of the ovary. Before the integuments begin to appear, the megaspore mother cell can be distinguished by the size of its nucleus (figs. 5, 11). It is subepidermal in origin and larger than the surrounding cells of the nucellus. After increasing considerably in size (fig. 12), 2 divisions occur which result in the formation of a typical row of 4 megaspores (fig. 13), of which the innermost is the largest and the one destined to develop into the embryo sac.

The inner integument begins to develop first (fig. 7, *ii*), but the outer (*oi*), which appears a little later, grows the more rapidly. It has been stated by POISSON (**16**), working on *E. Lathyrus* and *E. Peplis*, and by SCHWEIGER (**20**), investigating many species of *Euphorbia*, that the outer integument develops before the inner. It would be easy to arrive at a similar conclusion in the case of *E. Preslii*, since one rarely obtains a preparation showing the stage at which the inner integument is appearing at the base of the nucellus before any trace of the outer is to be seen, and since the outer integument grows so rapidly that it very early extends beyond the inner. In all probability, closer study of this species would show that in these also the inner integument begins its development first, as is the case in *E. corollata*, *E. Preslii*, and *E. splendens*.

At the time of the first nuclear division in the functional megaspore, the outer integument reaches about half-way to the tip of the nucellus; the inner integument is still extremely small (fig. 8). As the inner integument begins to grow more rapidly, the obturator first appears as a small swelling on the placenta (fig. 8, *ob*). Its cells increase in number (fig. 10, *ob*), the outer ones becoming long and slender and giving to the structure a very irregular outline. The nucellus grows out into a long beak (fig. 10, *n*) which extends beyond the integuments. At this time, the developing embryo sac has reached the 8-nucleate stage, and the obturator completely fills the space between the beaklike prolongation of the nucellus,

the placenta, and the ovary wall. The outer integument always extends considerably beyond the inner, even at the maturity of the embryo sac (fig. 10). The sac becomes deeply imbedded in the cells of the nucellus (figs. 9, 10). It is very long, and averages about 5 or 6 μ in thickness.

The functional megaspore grows considerably (fig. 14) before the first division of its nucleus. The other 3 megaspores disintegrate, but are visible at least as late as the 4-nucleate stage of the embryo sac as small, dark-staining cells at the micropylar end of the sac (fig. 17). The 2 nuclei resulting from the first division are usually to be found near the respective ends of the sac (fig. 16). In one case observed (fig. 17), however, one nucleus of each pair had moved nearer the center of the sac. That the latter case is exceptional is indicated by the fact that the 8 nuclei formed by the third division lie in 2 groups of 4 each at the respective ends of the sac. Cell division now occurs in the typical way (fig. 15). The synergids are oval in shape and each has a characteristic vacuole below the nucleus. The egg extends farther toward the center of the sac than the synergids. The 3 antipodals are well defined, angular cells, each with a conspicuous vacuole. After cell division is completed, the polar nuclei remain for a time in what seem to be their original positions near the respective ends of the sac (fig. 18). In one case they were found to have moved nearer the center of the sac (fig. 19), but no case was observed in which they had come in contact with each other previous to fertilization. MODILEWSKI (**13**) found no evidence of a fusion of the polar nuclei in *E. virgata*, which has a typical embryo sac of 8 nuclei. HEGELMAIER (**6**) found no fusion of either male or female nuclei, or of polar nuclei, in *E. dulcis*. I found no case showing actual fertilization. Fig. 19 shows the antipodal cells and the synergids apparently disintegrating, but the polar nuclei have not yet fused, and the egg nucleus shows no evidence of fusion with a male nucleus. This history differs from that observed by Miss LYON in *E. corollata*, where the antipodal nuclei are ephemeral and were observed but once, and where the polar nuclei fuse soon after their formation.

It is difficult to trace the course of the pollen tubes, should they be present, because of the long cells of the obturator. There seems

to be little possibility for self-pollination, for the neck of the cyathium is very small and the staminate flowers do not extend above the surface of the cyathium. When the embryo sac is mature, the staminate flowers are still rudimentary. Insect pollination is improbable because of the smallness of the cyathium and the smallness of the opening. Seeds are formed with marked regularity, which would hardly be the case if wind pollination occurred. HEGELMAIER (**6, 7**) found the same conditions with regard to pollination in *E. dulcis*.

Embryo

The fertilized egg divides in a plane parallel with the long axis of the sac (fig. 20). The second division occurs at right angles to the plane of the first (fig. 21). Further divisions result in the formation of a globular mass of cells (figs. 22, 23, 24). In all cases observed, the embryo formed no suspensor. The beak of the nucellus and the obturator gradually disintegrate as the embryo is formed, and the inner and outer integuments grow so as nearly to fill the large opening originally constituting the micropyle, but still leaving a small opening (fig. 25).

As early as the 2-celled stage of the embryo, endosperm nuclei appear at either side of the embryo. In the case represented in fig. 20, one endosperm nucleus (*en*) lies between the embryo and the micropylar end of the sac. The endosperm nuclei increase rapidly in number and are distributed quite uniformly throughout the peripheral region of the sac (fig. 24). Cell division does not occur in the endosperm until the embryo has come to consist of several hundred cells. The endosperm gradually fills the space originally occupied by the nucellar tissue (fig. 25, *n*).

The embryo changes as it grows from a globular (fig. 26) to an elongated form (figs. 28, 29). Fig. 30 shows the earliest stage at which cotyledons were observed. A well developed root cap is present in the mature embryo (figs. 27, 31, *rc*). When mature, the embryo (figs. 27, 31) is straight and its length nearly equals that of the seed, the root cap (*rc*) being pressed closely against the micropyle. Surrounding the embryo, except at the tip of the root cap, is the endosperm (fig. 27, *end*), whose cells contain a large amount of reserve food material in the form of starch, fat, and aleurone grains.

EUPHORBIA SPLENDENS

Embryo sac

The flowers of this species develop just as do those of *E. Preslii*, and there is also a similarity in the general structure of the obturator and the ovule. As in *E. Preslii*, the megaspore mother cell may be distinguished before the integuments have begun to develop (fig. 33). The mother cell is easily recognizable by its size, being several times as large as the surrounding cells of the nucellus, and by the fact that it contains a very large nucleus. It is situated 3 layers beneath the epidermis, and although in numerous cases I have found it only in this position, it is probable that in this species also it originates as a subepidermal cell and that the cells of the nucellus above it divide so that it comes to lie more deeply in the nucellus. After the mother cell (fig. 34) grows and elongates, and its nucleus also grows considerably, the latter prepares for division. At the time of the synaptic stage (figs. 35, 36), the nucleus has moved to the micropylar end of the cell. One unusually favorable preparation showed a late anaphase of the heterotypic division (fig. 37). The spindle in this figure occupies a central position and its long axis lies in the plane of the long axis of the nucellus. There are 12 small, nearly spherical daughter chromosomes in each of the 2 groups on the spindle.

Although the formation of the row of 4 megaspores was not observed, it is certain that 4 are formed, for when the functional megaspore has increased in size (fig. 38), 3 dark-staining masses can be distinguished at its micropylar end. This stage agrees with the corresponding one in *E. Preslii*, in which it is plainly the innermost of the 4 megaspores that develops into the embryo sac. This megaspore (fig. 38), even at the division of its nucleus, is not as large as the megaspore mother cell. In one case (fig. 39) 2 developing megaspores were found lying parallel to each other with their long axes parallel to the long axis of the nucellus. Each has a dark-staining mass of apparently 3 disintegrating cells at the micropylar end, but the number of these cannot be distinguished with certainty. This occurrence of 2 functional megaspores is doubtless very unusual, for it was observed in but one ovule.

The developing embryo sac becomes deeply imbedded in the nucellar tissue. After some growth of the functional megaspore, its nucleus divides (fig. 40) and one daughter nucleus moves to each end of the cell. At this stage further growth takes place, and while the first and second divisions of the nucleus (figs. 40, 41) are taking place, a large central vacuole is formed which persists for some time. An 8-nucleate stage was not found. In two cases 4 nuclei were found at the micropylar end (fig. 42), but only 3 at the antipodal end of the sac. In most cases in which the egg apparatus had been differentiated, no nuclei were to be found at the antipodal end of the embryo sac, indicating that the antipodal nuclei (and cells, if formed) must be ephemeral, unlike the antipodal cells of *E. Preslii*. Fig. 43 shows a sac with the egg apparatus fully formed and the polar nuclei apparently about to fuse near the egg, while the antipodal end of the sac shows 2 daughter nuclei of a recent division, with a cell plate between them. It is possible that after the second nuclear division in the developing megaspore, one of the two nuclei at the antipodal end divides some time before the other. One of the daughter nuclei of this (the third) division might then move to the micropylar end and function as a polar nucleus, its sister nucleus disintegrating before the remaining nucleus in the antipodal end (a daughter nucleus of the second division) finally divides. This explanation would also fit in with the condition found in the sac shown in fig. 42, which had only 3 nuclei at the antipodal end.

Fig. 44 shows another peculiar embryo sac in which there are plainly 8 nuclei at the antipodal end of the sac, at least 3 of which are surrounded by cell membranes. In this case, it is conceivable that each of the 4 nuclei at the antipodal end of the sac has divided and that none has as yet disintegrated. In all but these cases, the antipodal nuclei (or antipodal cells) had disintegrated. The egg apparatus seems to be quite typical (figs. 43, 45). At first the nucleus of the egg occupies the center of the cell, but later, as the egg grows, the nucleus moves to the side of the cell farthest from the micropyle. The egg is spherical, and its nucleus is not, in general, larger then the nuclei of the synergids. In the sac shown in fig. 45 one of the polar nuclei lies close to the egg, while the other seems

to be moving along the side of the sac toward the egg. The polar nuclei are at first not as large as the other nuclei of the sac, but before their fusion (fig. 46) they increase in size, each becoming larger than the egg nucleus. Fusion takes place below the egg in a plane either at right angles to (fig. 46) or parallel with (fig. 47) the long axis of the sac. The 2 nucleoles persist in the fusion nucleus until fertilization takes place.

The embryo sac of *E. splendens* differs from that of *E. Preslii* chiefly in the history of the antipodal cells, which in the latter species persist for some time after their formation; another difference is that in *E. Preslii* the polar nuclei remain in their original positions until after cell division occurs.

If fertilization does not occur immediately after the fusion of the polar nuclei, the synergids disintegrate, leaving the egg and the fusion nucleus close together at the micropylar end of the sac. In 2 embryo sacs in which fertilization was observed, the synergids were disintegrating, but their nuclei were still recognizable as dark-staining masses. In the sac shown in fig. 48, the pollen tube has destroyed one of the synergids and discharged one male nucleus, which may be seen in contact with the nucleus formed by the fusion of the polar nuclei. The latter nucleus still shows 2 nucleoles and is considerably larger than the egg nucleus. It has already moved a little way toward the antipodal end of the sac. The male nucleus also possesses 2 nucleoles and is crescent-shaped. The second male nucleus is a dark-staining mass still in the pollen tube and little more than its nucleole can be distinguished. It is about to pass down to the egg nucleus, the tip of the tube being within the cell membrane of the egg. Fig. 49 shows another pollen tube which has not yet discharged its male nuclei. It contains densely staining material which seems to be aggregated into several masses, but no nuclei are distinguishable. The polar nuclei in this case have not completely fused and the egg nucleus is in a resting stage. The pollen tube could not be traced back into the micropyle in either case, for only its tip seems to contain material that stains densely. With the triple stain, the contents of the tip of the tube always take up the safranin. Fertilization does not take place at the same time in the 3 ovules within the same pistil. A pollen

tube may be seen in each of them, but in one case it may be discharging its nuclei, while in another case the endosperm nucleus may have undergone several divisions. Figs. 48 and 50 were drawn from 2 embryo sacs within the same ovary.

Embryo

The fertilized egg does not divide immediately after the fusion of the nuclei within it. Usually there are 5 or 6 nuclear divisions in the endosperm before division of the egg occurs. In the sac shown in fig. 50, there are 4 endosperm nuclei, the egg still being undivided. The first division of the egg (fig. 51) is at right angles to the long axis of the nucellus. The developing embryo forms a short suspensor which is several cells in diameter. The terminal cell divides by a longitudinal wall after the embryo is about 4 cells in length (fig. 52). There seems to be nothing definite about the planes in which later walls are formed (figs. 53, 54), but a more or less globular mass of cells is formed at the end of the short suspensor (figs. 55, 56). In the embryo represented in fig. 56 the suspensor seems to be disintegrating. The mature embryo has a structure similar to that of *E. Preslii*, there being a well differentiated root cap and epicotyl.

Summary

1. The cyathium of both species studied begins as a papilla which arises between two bracts. The order of appearance of the parts of the cyathium is as follows: staminate flowers, involucre, ovules, carpels, and secondary staminate flowers which arise as branches of the first set.

2. The megaspore mother cell is subepidermal in origin in *E. Preslii*, and probably also in *E. splendens*.

3. An axial row of 4 megaspores is formed, the lowest of which develops into the embryo sac; the other 3 spores disintegrate.

4. The inner integument begins to develop before the outer, but the latter grows rapidly and soon overtops the inner.

5. The mature embryo sac is long and narrow, and is deeply imbedded in the tissue of the nucellus. In *E. Preslii* it has the

structure usual in angiosperms. In *E. splendens* there are peculiarities in the history of the antipodal nuclei which require further study to make definite conclusions possible. It seems probable that each of the 4 antipodal nuclei may undergo a second division.

7. The obturator arises as an outgrowth of the placenta. It fills the space between the beaklike prolongation of the nucellus, the placenta, and the ovary wall. Its cells disintegrate after the embryo begins its development.

8. At about the time of the first division of the egg of *E. Preslii*, endosperm nuclei come to lie between it and the micropylar end of the embryo sac.

9. The embryo becomes a round mass of cells; this mass elongates and later 2 cotyledons and a well developed root cap are formed. The mature embryo is straight, and, except at the tip of the root cap, is surrounded by the endosperm. In *E. Preslii* no suspensor was observed; in E. *splendens* there is a short suspensor.

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EXPLANATION OF PLATES XIV–XVI

All drawings were made with an Abbé camera lucida at table level, and Leitz oculars and objectives. The following combinations were used: ocular 4, objective 3, tube length 170 mm. (×200), figs. 1–10, 24, 28–31; ocular 4, objective 6, tube length 170 mm. (×800), figs. 11, 23; ocular 4, oil immersion 1/16, tube length 170 mm. (×2000), figs. 12, 13, 22, 33, 41, 45–47; ocular 4, oil immersion 1/16, tube length 212 mm. (×2600), figs. 14–21, 34–40; ocular 1, objective 3, tube length 140 mm. (×100), figs. 25, 27, 32; ocular 4, oil immersion 1/16, tube length 140 mm. (×1700), figs. 42–44, 48–56.

The following abbreviations are used: *b*, bract; *c*, carpel; *col*, cotyledon; *en*, endosperm nucleus; *end*, endosperm; *i*, integument; *ii*, inner integument; *in*, involucre; *n*, nucellus; *o*, ovule; *ob*, obturator; *oi*, outer integument; *p*, papilla; *rc*, root cap; s^1, staminate flower; s^2, secondary staminate flower; *sg*, stigma.

In all figures the micropylar end is at the top of the drawing; figs. 18, 19, 21, 42–50 are reconstructed from 2 or 3 sections each.

PLATE XIV

Euphorbia Preslii.—FIG. 1.—Appearance of a papilla which will develop into a cyathium.

Fig. 2.—Staminate flowers and involucre developing at base of papilla.

FIG. 3.—Ovules appearing at top of papilla, and carpels, staminate flowers, and involucre at base; at the left, a secondary staminate flower.

FIG. 4.—Longitudinal section of cyathium, showing 2 ovules within the ovary, and developing staminate flowers at base of pistillate flower; integuments have not yet appeared.

FIG. 5.—Nucellus, containing megaspore mother cell, but with no integuments as yet.

FIG. 6.—Inner integument just appearing at base of nucellus.

FIG. 7.—Inner and outer integuments at base of nucellus; embryo sac is developing.

FIG. 8.—Obturator appearing on placenta, and outer integument overtopping inner.

FIG. 9.—Obturator pushing up to nucellus.

FIG. 10.—Mature embryo sac deeply imbedded in tissue of nucellus, which has developed a beak; the long cells of obturator have filled space between nucellus and placenta.

FIG. 11.—Nucellus, showing megaspore mother cell.

FIG. 12.—Megaspore mother cell before division.

FIG. 13.—Axial row of 4 megaspores.

FIG. 14.—Functional megaspore, now deeply imbedded in nucellar tissue.

FIG. 15.—Binucleate embryo sac.

FIG. 16.—Four-nucleate embryo sac.

FIG. 17.—Four-nucleate embryo sac, with 2 of nuclei near center of sac; an unusual condition.

FIG. 18.—Mature embryo sac.

FIG. 19.—Polar nuclei have moved nearer center of sac; synergids and antipodal cells seem to have disintegrated.

FIG. 20.—Two-celled embryo, with endosperm nuclei; one of latter between embryo and micropylar end of sac.

FIG. 21.—Four-celled embryo; several endosperm nuclei between embryo and micropylar end of sac.

FIG. 22.—Embryo a globular mass of cells.

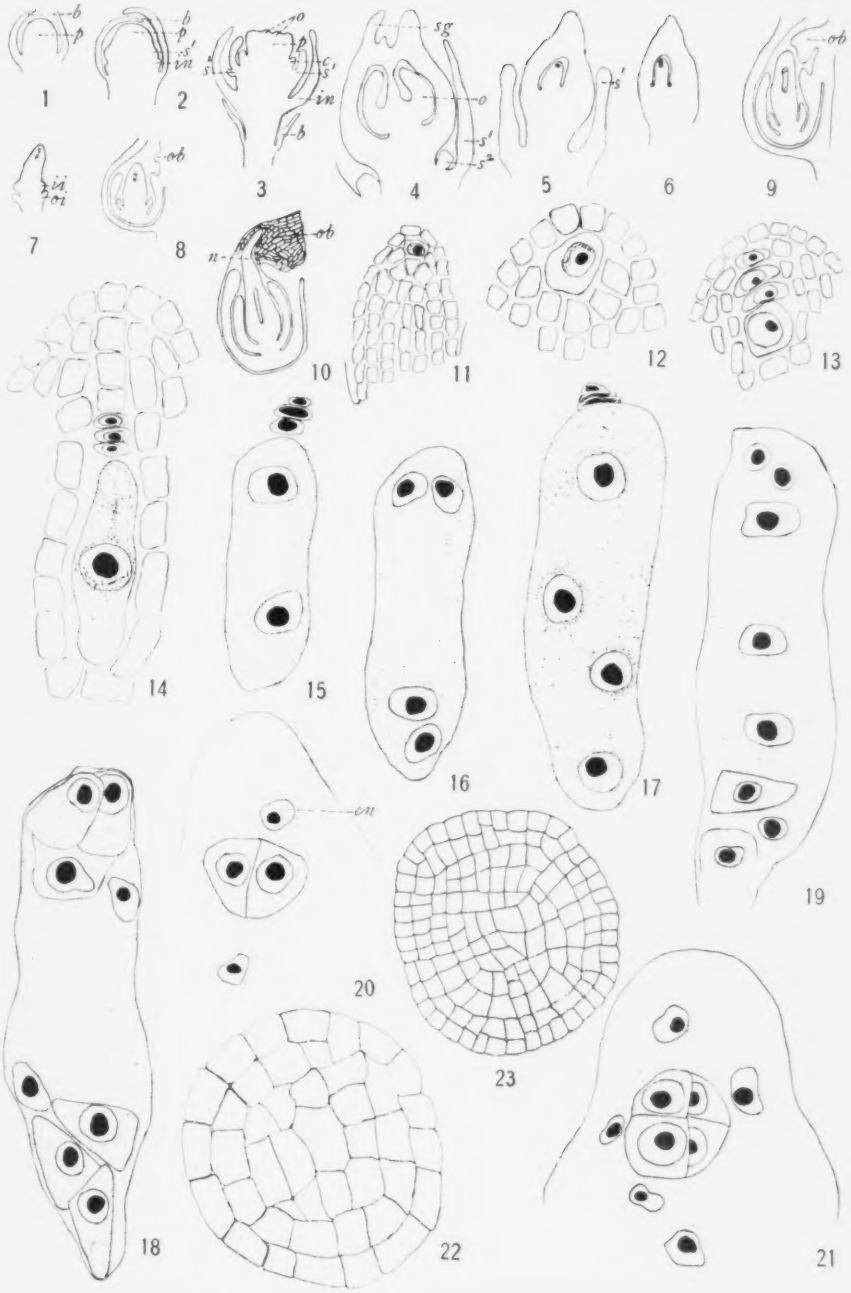
FIG. 23.—Still later stage in development of embryo.

PLATE XV

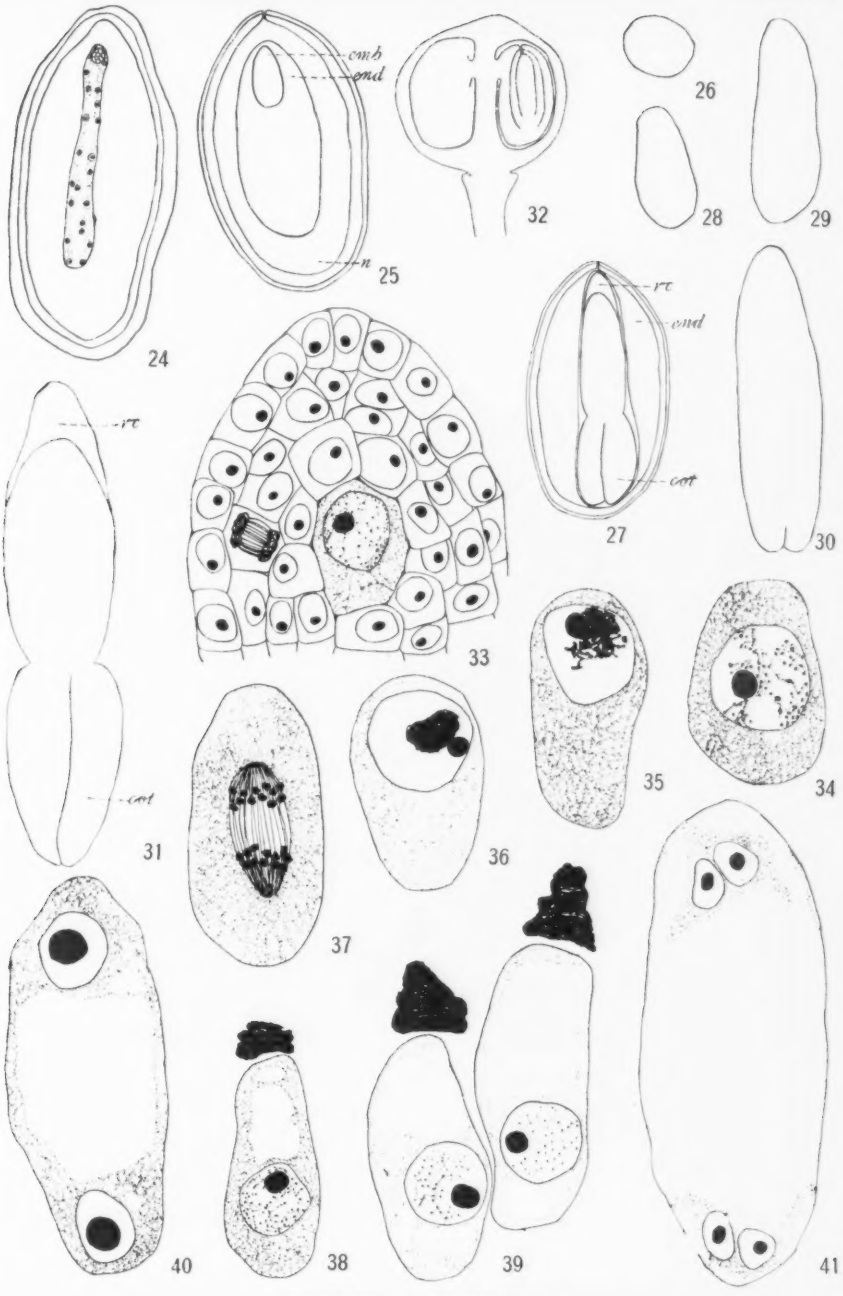
E. Preslii.—FIG. 24.—Longitudinal section of seed, with embryo at micropylar end of embryo sac, and endosperm nuclei distributed in peripheral region of sac.

FIG. 25.—Elongated embryo imbedded in endosperm.

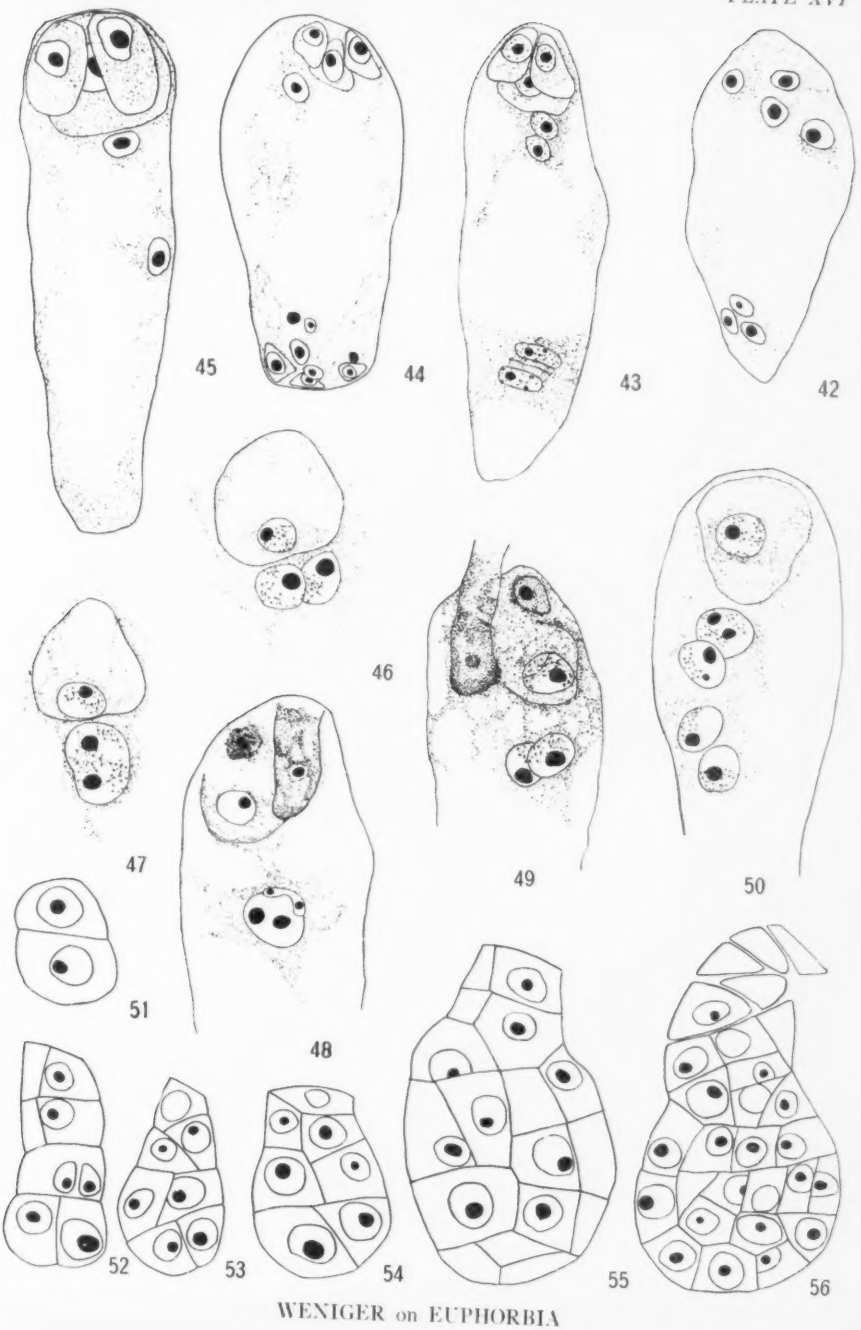
FIGS. 26, 28, 29, 30.—Elongation of embryo and appearance of cotyledons.



WENIGER on EUPHORBIA



WENIGER on EUPHORBIA



WENIGER on EUPHORBIA

FIG. 27.—Mature seed in longitudinal section.

FIG. 31.—Mature embryo.

FIG. 32.—Pistillate flower, with ovary jointed to pedicel.

E. splendens.—FIG. 33.—Nucellus with megaspore mother cell 2 layers below epidermis.

FIG. 34.—Megaspore mother cell with resting nucleus.

FIGS. 35, 36.—Synapsis in megaspore mother cell.

FIG. 37.—Anaphase of heterotypic division in megaspore mother cell.

FIG. 38.—Lowest of the 4 megaspores, which is to develop into embryo sac; other 3 megaspores have disintegrated.

FIG. 39.—Two functional megaspores side by side, each accompanied by what appear to be 3 disintegrating megaspores.

FIG. 40.—Binucleate embryo sac.

FIG. 41.—Four-nucleate embryo sac.

PLATE XVI

FIG. 42.—Embryo sac with 4 nuclei at micropylar end, and only 3 at antipodal end.

FIG. 43.—Embryo sac showing egg apparatus fully formed; 2 polar nuclei about to fuse near micropylar end; 2 daughter nuclei with cell plate near antipodal end.

FIG. 44.—Embryo sac with egg apparatus and 1 polar nucleus at micropylar end; 8 nuclei at antipodal end, 3 of which are inclosed by cell membranes.

FIG. 45.—Polar nucleus from antipodal end approaching other polar nucleus which lies close to egg apparatus.

FIG. 46.—Polar nuclei about to fuse near egg.

FIG. 47.—Polar nuclei fused, but 2 nucleoles still persisting; egg nucleus at one end of cell.

FIG. 48.—Fertilization: one male nucleus in contact with nucleus formed by fusion of polar nuclei; other male nucleus is still in pollen tube.

FIG. 49.—Pollen tube within embryo sac; no evidence of fertilization, and male nuclei not distinguishable within pollen tube; polar nuclei about to fuse.

FIG. 50.—Fertilized egg still undivided; 2 nuclear divisions have occurred in endosperm.

FIG. 51.—First division of egg.

FIG. 52.—Young embryo with terminal cell divided by longitudinal wall.

FIGS. 53, 54.—Later stage in embryo development.

FIG. 55.—Embryo consists of rounded mass of cells at end of a short suspensor.

FIG. 56.—Embryo has increased in size, and suspensor is beginning to disintegrate.

THE DEVELOPMENT OF THE ASCOCARP OF RHIZINA UNDULATA FR.

HARRY M. FITZPATRICK

(WITH PLATES XVII AND XVIII)

Our knowledge of the earliest stages in the development of the fruit body in the Helvellales is restricted to a limited number of species of the Geoglossaceae and one species of the Helvellaceae. Practically nothing is known of the ontogeny of any member of the Rhizinaceae. The question of the origin of the hymenium in this family, therefore, is of considerable interest.

Until recently it was believed that the ascocarp in members of the Helvellales, even in the youngest stages, is not covered by an inclosing membrane. In the system of classification employed by SCHRÖTER (**27**) the Helvellales are separated from the other orders of the Discomycetes on the basis of the gymnocarpous origin of the fruit body. The statement that members of this group are never angiocarpous, however, was evidently based upon general observations rather than upon careful study of young ascocarps, and subsequent investigations have demonstrated its falsity. The first evidence of the presence of a veil in this group was presented by DITTRICH (**10**) in connection with investigations on the Geoglossaceae. He discovered that in the youngest stages the fruit body of *Leotia lubrica* is inclosed by an envelope comparable to the volva of the Agaricaceae. This membrane later gelatinizes and is ruptured by the expansion of the ascoma within. Observations made by him on *Mitrula phalloides* disclosed a similar condition in that species. His collections of fruit bodies of representatives of the Helvellaceae, including species of *Helvella* and *Gyromitra*, revealed, however, no stages young enough to shed light on the question of the presence or absence of a veil in the beginning.

DURAND (**14**) in his monograph of the Geoglossaceae of North America reviews the work of DITTRICH on the development of *Leotia* and *Mitrula* and states that observations of his own on several different species point unmistakably to the same conclusion.

He has found a veil in *Spathularia velutipes, Mitrula phalloides, Microglossum viride,* and *Cudonia lutea.* The most conspicuous veil seen is present in *Cudonia lutea* and *Spathularia velutipes.* It persists in both species until the plants are one-third or even one-half grown, when it fragments into irregular pieces and falls away. DURAND publishes photographs showing very clearly the dehiscing membrane on the hymenium of maturing plants. He examined, however, very young ascomata of *Geoglossum glabrum, G. difforme,* and *Trichoglossum velutipes* without finding any trace of such a membrane. Finally, he expresses the opinion that "when the development of the Discomycetes shall be better understood it will be found that in none of them, not even in the Helvellaceae, is the hymenium exposed from the first."

More recently MCCUBBIN (**25**) has studied the development of the fruit body in *Helvella elastica,* and states that, in the earliest stages, the young ascocarp is inclosed by an envelope which later dehisces and completely disappears. He presents photomicrographs showing in median longitudinal section a single young fruit body bearing at the periphery an adhering bit of tissue, and says that this is a fragment of the transitory veil which earlier enveloped the ascocarp. Although his discussion covers stages earlier than that figured, his photomicrographs are unconvincing.

BROWN (**4**) has studied the development of *Leotia lubrica* and *L. chlorocephala,* but his account contains no information bearing on the question of the presence or absence of a veil in the early stages. CARRUTHERS (**7**) describes at considerable length the cytology of *Helvella crispa,* but does not attempt to study the earliest stages in the development of the fruit body. MASSEE (**24**) in his monograph of the Geoglossaceae makes no mention of the occurrence of a veil on any of the species in this family. So far as the writer is aware, no other investigation on the development of the fruit body in this order has been undertaken. No representative of the Rhizinaceae has been studied in the young condition.

Considerable difficulty is experienced in obtaining the youngest stages of the fruit bodies of members of the Helvellales, either by collection or culture, and it is not surprising therefore that students of the fungi have given little attention to developmental

studies in this group. Moreover, the number of species included in the Rhizinaceae is not large, and collections of some of these are rarely made (UNDERWOOD **29**, HONE **22**, BURT **6**).

During the summer of 1914 the writer discovered an abundant supply of the apothecia of *Rhizina undulata*, and was able to collect numerous very young fruit bodies in addition to the older stages. These have furnished all the necessary material for a thorough study of the development of the fruit body in this species. *Rhizina undulata* is particularly suitable for investigation, since it is the type of the genus and family, and probably the best known member of the group.

SCHRÖTER (**27**) separates the Rhizinaceae from the Geoglossaceae and Helvellaceae on the basis of the sessile fruit body. BOUDIER (**3**), attempting to arrange the Discomycetes in a natural classification, has developed a system very different from that of SCHRÖTER. He makes his primary separation on the basis of the method of rupture of the ascus. He places in one large group (Operculés) those forms whose asci open by an apical lid, and in the other group (Inoperculés) those whose asci open merely by a pore. By this separation the Helvellaceae and Rhizinaceae fall in the first group and the Geoglossaceae in the second. BOUDIER regards the Rhizinaceae as more closely related to such genera as *Peziza*, *Aleuria*, and *Sarcoscypha* of the Pezizales than to either the Helvellaceae or Geoglossaceae. LAGARDE (**23**) makes the primary separation also on the method of rupture of the ascus.

The facts brought out in the study of the development of the fruit body in various genera of the Discomycetes are especially interesting for the bearing they have on the questions involved in these two opposing systems of classification. The present investigation is undertaken with the hope that more complete information with reference to ontogeny will render less difficult the consideration of the phylogeny of the group.

Rhizina undulata Fr.

HISTORICAL.—The genus *Rhizina* was founded by FRIES (**16**) in 1815. It is characterized by the possession of prominent rope-like strands of mycelium, termed rhizoids. These are developed

in considerable numbers on the lower surface of the fruit body, and serve to attach it to the substratum. Representatives of this genus, therefore, are not easily mistaken.

Rhizina undulata was apparently first described by SCHAEFFER (**26**) under the name *Elvela inflata*. This writer published a colored figure of the plant which illustrates well the more evident characters of the species. FRIES (**16**) later described the fungus as *Rhizina undulata*, and discusses it under this name in *Systema mycologicum* (**17**). In accordance with the international rules of botanical nomenclature the writer designates the species by this name, but it has more commonly been referred to in recent literature as *Rhizina inflata*. The plant has been described by many writers and has frequently been figured. Excellent colored plates are given by BOUDIER (**2**). On account of the fact that the fungus is parasitic on the roots of certain trees its morphology and life history have received considerable attention (TUBEUF **28**).

HARTIG (**19, 20, 21**) discusses at some length the structure of the mature fruit body. He made no attempt to study its development. More recently WEIR (**30**) has published photographs of apothecia with notes on the parasitism of the fungus. None of these workers describes other than the mature condition.

MATERIAL AND METHODS.—The apothecia used by the writer in these investigations were collected in July 1914 in a small pine wood north of Beebe Lake near the Cornell University campus at Ithaca, New York. Due to favorable weather conditions the fruit bodies were developing in great profusion, and dotted the ground throughout a considerable portion of the wood. Although no attempt was made to obtain corroborative evidence as to the parasitism of the species, it was noted that the fruit bodies in many cases were firmly attached to the roots of living pines. Transverse sections through pine rootlets will be noted in the accompanying plates. In fact, the youngest fruit bodies were obtained more easily by tearing up a superficial root invested with wefts of mycelium on which the young fruit bodies were being differentiated. In this manner immature fruit bodies in all stages of development were obtained easily. Mature apothecia were available in such abundance that several quart jars of material were preserved for class

use. The writer's determination of the fungus was confirmed independently by E. J. DURAND and F. J. SEAVER, and his thanks are due both of these gentlemen.

The young apothecia collected for the study of the development of the fruit body were immediately placed in medium strength chromo-acetic acid fixer. They were carried into paraffin, and were studied in serial sections of 4–7μ thickness. The material was stained chiefly with Heidenhain's iron alum-haematoxylin, no counter stain being used. For certain of the more mature stages the shortened Flemming's triple stain proved more useful. The material was sectioned and stained in the laboratories of the Brooklyn Botanic Garden in the summer of 1915, while the writer held a visiting fellowship at that institution. He wishes to take this opportunity to express his appreciation of the courtesy of Director C. S. GAGER in extending to him the facilities of the laboratories, and to acknowledge his indebtedness to Dr. E. W. OLIVE for many kindnesses, including several helpful suggestions concerning technique. The investigation was carried to completion in the laboratories of the Department of Plant Pathology at Cornell University.

THE MATURE FRUIT BODY.—The mature apothecia exhibit great variation in size and shape. Considerable irregularity of contour is characteristic, and the early fusion of several fruit bodies results at maturity in large unsymmetrical structures. The apothecia shown natural size in fig. 1 illustrate well the extent of variation. The two fruit bodies in the lower left hand corner of the figure were inverted to reveal the clusters of ropelike rhizoids which give to the genus its name. The fruiting surface varies from a rich chestnut to a dark brown, and when moistened is peculiarly sticky and glutinous. Around the margin of the apothecium a sterile zone is indicated by a narrow, white, encircling band which contrasts sharply with the brown hymenium. This white margin is very evident in all stages. In the youngest fruit bodies the entire surface is white, the brown fruiting layer later making its appearance at the center and increasing rapidly in extent. The smaller of the fruit bodies pictured in fig. 1 show this condition clearly.

THE HYMENIUM.—The hymenium at maturity contains 3 types of structures: asci, paraphyses, and paraphysis-like structures which the writer will designate as setae, since they arise far below the hymenium, and are dark colored and thick-walled. The asci are narrow, cylindrical to clavate, and 8-spored. The spores are uniseriate, fusiform, hyaline, unicellular, and at maturity biguttulate. The paraphyses are filamentous, unbranched, multiseptate, hyaline, and at the apex distinctly clavate. The setae are heavy-walled, brown, non-septate, unbranched tubes originating far below the hymenium (fig. 11) and discharging a brown sticky secretion at their tips. This secretion flows over the surface of the hymenium made up of the swollen tips of the paraphyses, and gives a condition superficially resembling an epithecium. HARTIG (**20, 21**) states that it is impossible to procure a pure culture from the spores of this fungus on account of the bacteria which swarm in myriads in this glutinous secretion and find their way down between the paraphyses. These bacteria induce a rapid decay of the entire apothecium, and give to it in age a peculiar water-soaked, brittle consistency. In fig. 13 a portion of the hymenium is shown at a stage approximating maturity. The broad, deep-staining tubes are the setae. Surrounding these are the paraphyses, and pushing up from below may be seen the young, uninucleate asci. The swollen tips of the paraphyses are obscured by the layer of deep-staining glutinous material.

MYCELIUM.—The mycelium of *Rhizina undulata* possesses more than ordinary interest for the systematist. It is described by HARTIG (**21**) as bearing clamp connections. He says: "Although I have much diffidence in maintaining that this feature, which otherwise is peculiar to the Hymenomycetes, is characteristic of this parasite, still I cannot doubt that these filaments with clamp cells belong to it." The writer has given the mycelium careful examination, and has been unable in his collections to find clamp connections on hyphae certainly belonging to the fruit bodies of *Rhizina*. He does not feel, however, that sufficient investigation of this point has been carried on to enable him to state definitely that they never occur. The mycelium develops profusely, and covers the soil particles and small rootlets as a whitish, moldlike

growth. Upon this subiculum compact masses of hyphae develop as minute, snow white knobs. These represent the primordia of fruit bodies.

DEVELOPMENT OF THE ASCOCARP.—The youngest fruit body sectioned measures slightly less than 0.3 mm. in lateral diameter. A considerable number of others possess a maximum diameter of 1 mm. or less. The youngest fruit body studied (fig. 2) is a wholly undifferentiated "button" of mycelium. The hyphae making up the primordium arise in this case from about a small rootlet, and pushing upward between other rootlets run more or less distinctly parallel toward the surface of the ground, where they radiate in every direction, giving the primordium its rounded form. At this early stage there is no indication of sexual cells, and no evidence other than shape that this "button" of mycelium is to develop into a fruit body.

The hyphae at the surface of the primordium form a more or less definite palisade layer, although at this early period they are sufficiently flexuous to destroy the very definite palisade effect evident later. These hyphae in many cases can be traced backward with ease to the point of origin of the fruit body. No structure of the nature of an enveloping veil is present, and it is incredible that one could have existed at an earlier period. Neither in this nor in any later stage has the writer been able to find remnants of a ruptured envelope such as that figured by MCCUBBIN (**25**) for *Helvella elastica*. He has searched for these in sections of many very young fruit bodies and is absolutely convinced that in *Rhizina undulata* the ascocarp is at no stage provided with a veil. The fruit body is therefore gymnocarpous and the hymenium is "exposed from the first." Fig. 2 shows in median longitudinal section a fruit body of *Rhizina undulata* considerably younger than the youngest stage photographed by MCCUBBIN in *Helvella*. The deep-staining spots at the side and base of the primordium are transverse sections of pine rootlets.

MCCUBBIN states that in *Helvella elastica* "the envelope which covers the fruiting body in its early stages arises from the palisade layer. Many of the club-shaped hyphae of the latter continue to grow out beyond the general surface, then turn at right angles,

and interlacing in every direction along the surface form a matted web 2–8 threads in thickness. This membrane is very transitory, however, and undergoes degeneration at an early period. Its protoplasm takes on a granular appearance, the cell outlines become indistinct, and finally the whole disintegrates into a deeply staining mass in which the nuclei are the most prominent feature. Long before the process is complete, however, the rapid growth of the underlying tissue bursts the envelope so that it adheres in flakes (figs. 57, 58). Then the paraphyses and intercalary palisade hyphae pushing out to the surface complete the separation and all traces of it are cast off."

It is to be regretted that McCUBBIN did not publish photomicrographs of stages younger than that shown in his fig. 57. If in *Helvella*, as he states, the envelope, which incloses the fruit body in the early stages, arises from the palisade layer, it might be concluded that the section of *Rhizina undulata* shown in fig. 2 is too young to possess the envelope, and that it might logically be expected to develop later on older fruit bodies. That it does not do so, however, is certain. The writer has had available a sufficiently large number of fruit bodies in all stages of development to preclude any misinterpretation with reference to this point. No veil or fragment of a veil has been found on any of the fruit bodies sectioned.

Figs. 3–6 show median longitudinal sections through primordia somewhat older than that pictured in fig. 2. The magnification in the 5 cases is the same, being 40 diameters. Other fruit bodies sectioned, of intermediate sizes, bring out no additional facts. In fig. 3 the palisade nature of the hyphal arrangement at the periphery is evident. The deep-staining area on the upper surface to the left of the center is a fragment of a sectioned rootlet other portions of which were cut away in trimming the print. Other sections of similar rootlets appear at different places in the interior of the fruit body. At the base of the ascocarp can be noted the tendency of the mycelium to form thick rhizoids. These young rhizoids appear in section in figs. 3, 5, and 6. Fig. 5 shows the palisade layer of hyphae very clearly. In fig. 10 a portion of the palisade layer of a fruit body approximately the same age as that

in fig. 5 is shown much enlarged. It will be noticed here that the tips of the hyphae at the periphery stain very deeply. This is probably due to the fact that, since growth is taking place much more rapidly at the tips of the hyphae, the protoplasm at this point contains as the result of metabolism more deeply staining contents.

In certain young fruit bodies (fig. 4) the setae are developed much earlier than in others. The reason for this is not known. They are prominent organs, originate from the deeper lying tissue of the fruit body, and protrude beyond the palisade layer as deep-staining spines. These are shown much enlarged in fig. 14. It will be noted that they are of much greater diameter than the other hyphae of the ascocarp. They arise as differentiations of ordinary vegetative hyphae.

SEXUALITY.—Near the center of the sections shown in figs. 3 and 5 are to be seen deep-staining elements. These bodies constitute the sexual apparatus of the fungus, and at a somewhat later stage (fig. 9) give rise to the ascogenous hyphae. Since the writer is engaged in the preparation of another paper dealing with the details of the sexual process in *Rhizina undulata*, he will refrain from further comment on these structures at this point.

PARAPHYSES.—The layer of paraphyses is developed comparatively early in the history of the fruit body and constitutes a well defined zone long before the asci are produced. Fig. 7 shows a median longitudinal section through a young apothecium on the upper surface of which the layer of paraphyses is being differentiated. In fig. 8 this same layer is shown more highly magnified. The paraphyses arise from the ordinary hyphae in the interior of the fruit body, and are in reality a specialized portion of the palisade layer. As the fruit body enlarges by the elongation and branching of the hyphae at the periphery, those palisade hyphae which lie on the upper surface increase in number, run more nearly parallel, and come to stand very close together. They soon constitute a well defined zone, the individual units of which appear straighter, slightly narrower, and many times more abundantly septate than the palisade hyphae covering the remainder of the fruit body. This layer of paraphyses continues to develop at the margin as the fruit body increases in diameter, the line of demarcation between

paraphyses and palisade hyphae at the point of contact never being very sharp. Fig. 9 pictures approximately one-half of a median longitudinal section through an older fruit body in which the layer of paraphyses has become sharply differentiated from the tissue of the fruit body below. The rounded sterile margin of the apothecium is here evident.

ASCOGENOUS HYPHAE.—Immediately beneath the paraphyses is a deeper-staining zone filled with the ultimate tips of the profusely branching ascogenous hyphae. These hyphae have their origin near the base of the fruit body in the sexual apparatus previously mentioned, and may be seen ramifying throughout the interior of the ascocarp as they branch and rebranch on their upward journey toward the hymenium. At this stage these threads have not yet undergone crozier formation at their tips, and no young asci are present. Fig. 12 shows a section through the hymenium of a more mature apothecium in which the young asci are pushing up among the paraphyses. The septate paraphyses, the tubular setae, and the young, deep-staining, clavate asci show here to good advantage. In fig. 13 the asci are shown at about one-half their mature size, and the fusion nucleus may clearly be seen in each. In this and other sections the deep-staining glutinous secretion previously discussed forms a well defined layer above the clavate tips of the paraphyses.

General considerations

The results of the present investigation on the origin and development of the ascocarp in *Rhizina undulata* are particularly interesting in the light of the facts disclosed by various workers on other allied forms. Before the publication of the work of DITTRICH (10) on the development of *Leotia lubrica* and *Mitrula phalloides*, it was generally assumed that in the 3 families of the Helvellales the fruit body is gymnocarpous. After the appearance of DITTRICH'S paper the pendulum of opinion swung to the other extreme, and we find the statement made by DURAND (14) that in his opinion "when the development of the Discomycetes shall be better understood it will be found that in none of them, not even in the Helvellaceae, is the hymenium 'exposed from the first.'" It is evident now from the results of researches on various

Geoglossaceae that certain members of this family are at first provided with a veil. It is equally certain that in *Rhizina undulata* no enveloping membrane is ever present. Both conditions occur therefore within the order. Whether it will prove possible to separate the families of the orders on the basis of the presence or absence of a veil is doubtful, but additional investigations on members of the 3 families will be necessary to determine this point. Since the work of McCUBBIN (**25**) on *Helvella elastica* is the only contribution of any importance to our knowledge of the development of the fruit body in the Helvellaceae, it is desirable that other representatives of this family be studied. Also, since McCUBBIN has stated definitely "from observations on a very complete series of stages that *Geoglossum hirsutum* shows no trace whatever" of a veil, it is desirable that photographs be published demonstrating the gymnocarpous nature of the ascocarp in this or other members of the Geoglossaceae in which a veil is absent at all stages. Finally, the development of the fruit body in additional species of the Rhizinaceae should be studied to determine whether the conditions described for *Rhizina undulata* are typical of the entire family.

It has become increasingly evident since the publication by SCHRÖTER (**27**) of his system of classification of the Discomycetes that his basis for the separation of the Helvellales from the other orders of the group is untenable. Not only has it been demonstrated that in certain of the Helvellales the fruit body is angiocarpous, but also in the Pezizales it has been shown that certain species possess a fruit body which is clearly gymnocarpous. As representatives of this latter group may be enumerated *Ascodesmis* (CLAUSSEN **8**), *Pyronema confluens* (HARPER **18**, CLAUSSEN **9**, et al.), *Lachnea stercorea* (FRASER **15**), *L. scutellata* (BROWN **5**), and *Ascobolus magnificus* (DODGE **11**, **12**, **13**). The presence or absence of a hyphal envelope, therefore, cannot be used to separate the Helvellales and Pezizales as constituted by SCHRÖTER, and some other system of classification of the Discomycetes must be employed. That of BOUDIER (**3**) has met with considerable favor.

As pointed out by DODGE (**13**) and ATKINSON (**1**), several well defined types of ascocarps are present in the Ascomycetes, and these

should be considered in any system of classification. The somewhat loose use of the terms "angiocarpous" and "gymnocarpous" and of the phrase "hymenium exposed from the first" has resulted, however, in some confusion. In some species (for example *Leotia lubrica*) the ascocarp is at the beginning inclosed by an envelope which is transitory and disappears before the hymenium is formed, while in others (for example, *Rhizina undulata*) it lacks at all stages any indication of a veil. In both cases the hymenium is "exposed from the first," but the development of the fruit body is essentially different, and if the veil has any phylogenetic significance the two forms cannot be regarded as closely related. DODGE (13) states that "the real question as to whether an ascocarp is to be classed as open or closed in its early stages depends upon whether the young hymenial layer arises endogenously, as in *Ascobolus furfuraceus*, or is from the first free and exposed, as in *Pyronema*." It seems to the writer of greater significance to determine whether the ascocarp is itself at any stage inclosed by an envelope. This is certainly true from the standpoint of phylogeny.

Summary

1. The mycelium of *Rhizina undulata* Fr. spreads among the soil particles, and covers the smaller rootlets of pines and other trees, forming a whitish moldlike growth. Upon this subiculum compact masses of hyphae develop as minute, snow white, rounded knobs. These constitute primordia of ascocarps.

2. The ascocarp primordium in the youngest stages shows no evidence of a sexual apparatus. It is made up of undifferentiated hyphae, which at its surface form a palisade layer.

3. The ascocarp is neither at the beginning nor at any subsequent period provided with a hyphal envelope. The fruit body is therefore gymnocarpous and the hymenium is "exposed from the first."

4. There is developed in the interior of the young ascocarp a well defined sexual apparatus from which the ascogenous hyphae arise. The details of the sexual process have been studied and will be described in a later paper.

5. The ascogenous hyphae branch repeatedly and undergo crozier formation in the development of the young asci.

6. The paraphyses are a differentiation of the palisade layer which covers the fruit body at all stages.

7. In the ascocarp of this species there are present paraphysis-like structures which arise early in the history of the fruit body. They are non-septate, thick-walled tubes which originate far down in the hypothecium, traverse the hymenium, and discharge a brown, glutinous secretion at their tips. The writer has applied to these the term "setae."

8. At maturity the ascocarp is variable in size and shape. The brown hymenium is bordered by a sterile white margin.

9. There are present on the lower surface of the ascocarp numerous prominent rhizoids.

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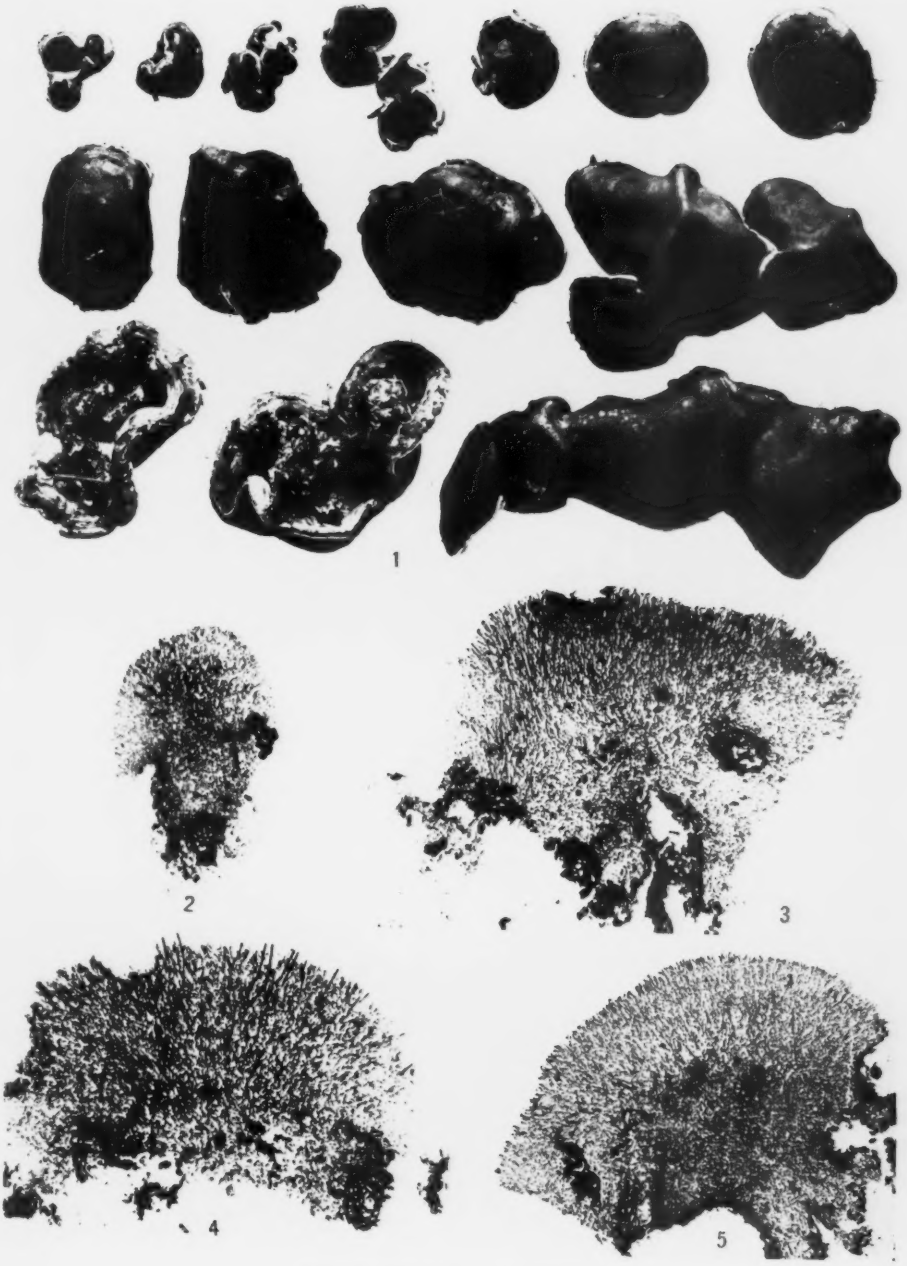
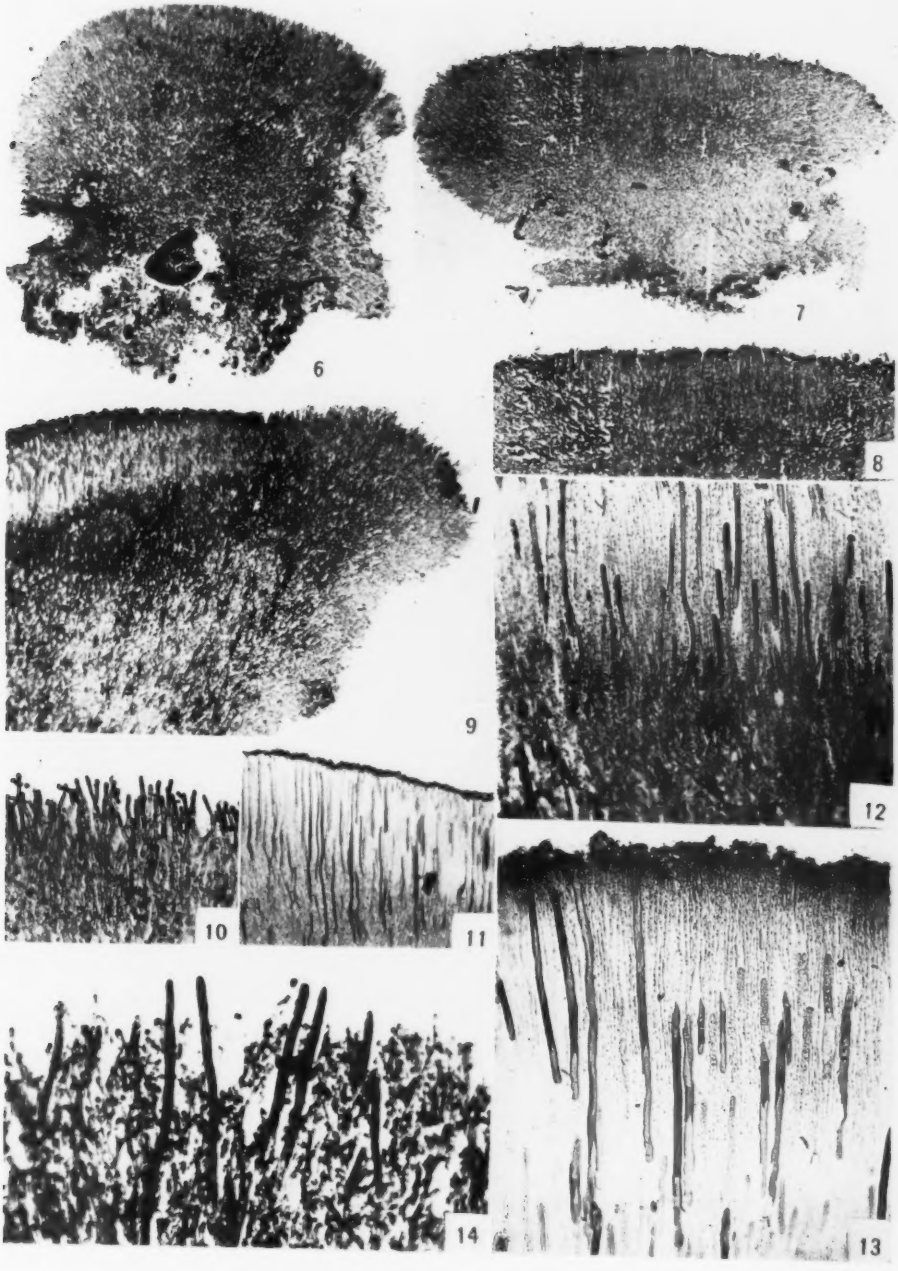


FITZPATRICK on RHIZINA



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EXPLANATION OF PLATES XVII AND XVIII

FIG. 1. *Rhizina undulata* Fr., natural size, Ithaca, New York, July 1914; group of apothecia selected to show variation in size and shape; note sterile white margin on both young and old plants, and tendency for adjacent fruit bodies to fuse; at lower left hand corner of figure 2 apothecia are inverted to show lighter colored, lower surface and dense clusters of stout rhizoids which serve to attach the fruit body to substratum.

FIG. 2.—Median longitudinal section through a very young ascocarp primordium, ×40; note pine rootlets in section at side and base.

FIG. 3.—Median longitudinal section through a somewhat older fruit body, ×40; deep-staining body at periphery above is fragment of section

through a pine root such as those shown in the lower half of fruit body; deep-staining structures near center of section are sexual cells which later give rise to ascogenous hyphae; at base young rhizoids are shown in section.

FIG. 4.—Median longitudinal section through a young fruit body in which setae have developed early, ×40; these may be seen projecting above layer of palisade hyphae.

FIG. 5.—Median longitudinal section through a young fruit body, ×40; palisade layer of hyphae at periphery shows plainly; note sexual cells at center of section.

FIG. 6.—Median longitudinal section through a slightly older fruit body, ×40.

FIG. 7.—Median longitudinal section through a somewhat older fruit body in which the layer of paraphyses is being differentiated from palisade layer, ×29.

FIG. 8.—Layer of paraphyses shown in fig. 7 enlarged to show structure more clearly, ×40; note indefinite line of demarcation between layer of paraphyses and palisade layer of sterile margin.

FIG. 9.—Approximately one-half of a median longitudinal section through considerably older fruit body, ×32; note well defined layer of paraphyses, sterile margin, and definite, deep-staining zone below the paraphyses made up of tips of ascogenous hyphae; ascogenous hyphae can be seen originating near base of apothecium and branching profusely as they ramify throughout the fruit body and approach hymenium.

FIG. 10.—Portion of section such as presented in fig. 5 enlarged to show structure of palisade layer, ×192; note deep-staining tips of hyphae.

FIG. 11.—Longitudinal section through young hymenium of fruit body of about the same age as that shown in fig. 9, ×192; note numerous prominent setae originating below hymenium; note also deep-staining layer at tips of paraphyses, resulting from glutinous secretion poured over hymenium by setae.

FIG. 12.—Longitudinal section through immature hymenium of fruit body somewhat larger than that shown in fig. 9, ×192; note slender, septate paraphyses, prominent tubular setae, and young deep-staining asci.

FIG. 13.—Longitudinal section through hymenium of fruit body approaching maturity, ×192; asci have not yet formed spores; fusion nucleus is visible in some cases.

FIG. 14.—Portion of section given in fig. 4 enlarged to show setae at margin of fruit body, ×192; note that they are much larger in diameter and more deeply staining than the hyphae of palisade layer.

PROBLEMS OF PLANT PATHOLOGY[1]

F. L. Stevens

Plant pathology is primarily and essentially an economic subject, and it is mainly from this viewpoint that it will be considered in this paper, attention being called also to the relation which the practice of pathology bears to science. The chief application of plant pathology is to agriculture, and as so applied the main, practical achievements may be summarized briefly as follows: (1) the control or partial control of various fungi, notably of orchard, vineyard, truck, and floral crops by sprays of copper compounds; (2) the substitution in many instances, notably on drupaceous hosts, of lime-sulphur compounds; (3) treatment by excision and the introduction of so-called tree-surgery; (4) the avoidance of susceptible varieties, for example, carnations, pears, strawberries, chrysanthemums, cowpeas, asparagus, and cantaloupes; (5) the development or utilization of disease-resistant or partially resistant strains, for example, asparagus, pears, watermelons, cowpeas, oats, wheat, flax, and cabbage; (6) the prevention of disease through knowledge of necessary alternate hosts, for example, pomaceous rusts; (7) the prevention of disease introduction by quarantine and inspection; (8) prevention through knowledge of mode or time of infection, or mode of transference, for example, certain cereal smuts, bean and cotton anthracnose, cabbage blackrot, and potato scab.

In this enumeration, the first 5 captions cover the major part of the early fruits of pathology, the easily gathered fruits, first ripe, and which could be harvested without deep, scientific knowledge. Such practices do not necessarily rest upon subtle principles, but are rather the outcome of cut-and-try methods of experience.

The accident which led to the experiments which in turn brought into prominence the use of copper sprays and thence led to much that has been done to perfect sprays and dust applications

[1] Paper presented at the Botanical Conference held in connection with the Quarter-Centennial Celebration of the University of Chicago, June 1916.

is well known; also the taking over of the lime-sulphur mixture from the entomologist. The susceptibility of certain varieties of crop plants has led the farmer in the south to reject special types; for example, the Bartlett and Seckel pears, and some of the strawberries. The florist, likewise, has been obliged to eliminate certain varieties of carnation. They would have done so had no science of pathology existed. Similarly, many of our so-called disease-resistant varieties were discovered by the farmer or are either not highly resistant or are of such poor quality that they are not used extensively.

Lest I may be regarded as pessimistic concerning the relation of science to plant pathology, I shall say at once that though these large advances were made in the main empirically, their progress was in reality hastened and made easier by the faith and confidence fostered by basic knowledge in mycology, bacteriology, and physiology; and definitely aided by advances in chemistry and special technique. With the remaining categories the dependence upon science is direct and evident. For example, knowledge of the alternate host relation in pomaceous rust and currant rust could not have been attained without the upbuilding of a broad knowledge of the rusts in general, indeed of the fungi as a whole; nor could the canker relation in apples (*Glomerella*, *Sphaeropsis*, *Bacillus*) have been found without knowledge of the nature and morphology of the causal fungi; and the same is true of the present troublesome citrus canker. Adequate quarantine inspection measures cannot be taken without definite knowledge of the identity, nature, and causes of disease. The quarantine question looms into particular prominence when we regard the subject of disease migration both interstate and international. The following are examples of diseases, the migration of which is more or less definitely known: pear blight, asparagus rust, grape anthracnose, cabbage club root, potato wart, potato blight, grape blackrot and downy mildew, chestnut bark disease.

Seed transference of disease is exemplified in the cabbage blackrot, celery leaf spot, and bean and cotton anthracnose. It is barely possible, but not at all probable, that this relation could have been discovered without intimate knowledge of the causal

agents, but it really was a rigid, scientific method which gave us our present knowledge of these diseases. The valuable results of the work on cereal smut infection furnish a fine example of achievement in disease-prevention that could not have been attained without both basic knowledge in mycology and a technique enabling trustworthy experimentation.

Upon entering a new biological territory, the first work is to collect and to classify, to know the material. So in the new field of plant pathology much of the early work was descriptive. The number of important plant diseases that are reasonably well described in two volumes of the Report of the United States Department of Agriculture for the years 1887 and 1888 is remarkable.

While the descriptive period in plant pathology is not entirely past, trivial diseases of cultivated plants, weeds, and wild plants still remaining undescribed, there have been very few really important diseases of general interest recently discovered in this country, few which compare in importance with the apple bitter rot, tomato leaf spot, onion smut, potato blight and scab, the cereal rusts or smuts. Many of the diseases recently described are of minor importance or are at present of very narrow geographic range; some have never been noted except by those who described them.

With the general principles of treatment established and the field for discovery of new diseases dwindling in importance, the time has now come when further progress, with rare exception, must be the outcome of fundamental, special knowledge and crucial experiments. It is evident that the easy crop from the virgin soil has been harvested, and that now we are entering upon the era of intensive cultivation.

The conquests of the future will be mainly the result of intensive study of the diseases and disease agents now known. Compare the degree of thoroughness of our knowledge of any one plant disease with any one disease in medicine. For example, compare from the research viewpoint our knowledge of *Pseudomonas campestris* with that of *Bacillus typhosis;* of the morbid histology of wheat rust with that of diphtheria; of the "epidemiology" of any plant disease with that of any human disease. Of course, the

parallel is not fair, since the values are not commensurate, but it serves to make the point that if such knowledge in medicine is probably contributory to prevention, probably it is also contributory in our science.

Thoroughness such as is attained in human pathology is in reality manifestly impossible for several reasons, one being the large number of plant diseases. Each plant species, to an extent, has its own fungous parasites; there are more than 40 listed for the apple alone. There are, perhaps, between 300 and 400 really significant, economic plant diseases, and to master knowledge of these is a great undertaking which is far from realization as yet.

Parasitic diseases present two chief elements, the host plant and the parasite. There is also, what is perhaps more important, the interrelation between these two, and what is also very important, the relation between these two and the factors of environment. It is with the study of these 5 elementary factors that pathology has to do. Large attention in the past has been given to the parasite, and in many cases it is the parasite alone which has been studied. Proportionately little study has been given to the relations existing between the host and the parasite, while the relations existing between environment and host, and environment and parasite, unquestionably of great significance, present a comparatively unworked field.

I wish to call attention briefly to the types of problems that exist under the above analysis. Perfecting and stabilizing of the taxonomy and nomenclature of the parasites are of course of fundamental value to pathology. The limitation of the families and orders of the fungi is, in the main, confessedly artificial; the boundaries of the genera are poor, and within the genera but little is really satisfying. To illustrate, the form genera *Penicillium* and *Coremium* are separated by ordinal rank, yet a single culture, dependent upon conditions, may give the characters of one or the other. Ordinal questions occur regarding *Meliola*, *Thielavia*, *Fusarium*, *Actinonema*, *Helminthosporium*, and many other genera.

Examples of problems in generic limitation are the *Phoma-Phyllosticta*, the *Septoria-Rhabdospora-Cylindrosporium*, the *Meliola-Capnodium-Apiosporium-Antennaria* questions. Within the

genus a good example is *Septoria* with 1200 species, or *Phyllosticta* with 1150 species. The former has nearly 700 species between 20 and 50 μ in spore length. The latter has 128 species with spores measuring 5–6 μ long. *Septoria* has 115 species on Compositae, and 77 species on the Gramineae (26 of these are within the limits of 20–40 μ in spore length).

Our present knowledge of such genera, as given by SACCARDO, is essentially that of a preliminary cataloguing of these forms by their hosts, the necessary first step. And we may add, as examples, the species of such genera as *Phoma*, *Rhabdospora*, *Cercospora*, *Nectria*, *Sclerotinia*, *Guignardia*, *Physalospora*, and *Phyllachora*. The bearing of this condition upon practice is evident, since numerous forms described as separate species upon the same economic host plant in reality may be identical or may be co-specific with forms described as distinct species or as belonging to other genera, families, or orders on the same or other hosts. The next step, well exemplified by such work as the monographs of THEISSEN, will consist in morphological comparison and readjustment of the species. This raises the question of life histories, of course, and shows the need of much such work as that of SHEAR and WOOD on *Glomerella*, HIGGINS on *Cylindrosporium* (*Coccomyces*), CLINTON on *Venturia*, WOLF on rose black spot (*Diplocarpon*), etc.

In connection with these problems arises the question of host relation and of biological specialization, as best exemplified, perhaps, in the rusts and the powdery mildews. What is the status of such specialization in the Fungi Imperfecti, in *Phyllosticta*, *Septoria*, *Cercospora*, etc., in the Ascomycetes, *Nectria*, *Sclerotinia*, *Phyllachora*, and many other genera? This forms a large and enticing field, in which much good work has been done, but a vast amount remains still to be done.

Coupled with these problems, come of necessity physiological, morphological, and cytological studies. The *Oospora-Actinomyces-Streptothrix* problem will require, apparently, all the possible sidelights before solution. This illustrates admirably the dependence of practice upon science, since fundamental questions of practice must rest their answer upon the degree of biological specialization

and variation of this organism, which causes potato scab, and concerning which we cannot decide as yet whether it belongs to the Eumycetes or to the bacteria.

Morbid histology of the various diseases presents a large field for activity. Concerning many diseases our knowledge in this regard is as yet really nil. It may be in many cases that such knowledge will not affect practice, but in many cases it surely has done so. Its utility appears clearly in relation to cereal smuts, tree surgery, etc.

The whole question of disease-resistance and susceptibility is fundamental and practical, as the age incidence of disease; the causes of resistance, whether mechanical, chemical, or physiological; the factors of air, soil, or heredity causing variation in resistance, and the possibility of artificially changing these factors. Breeding for disease-resistance is a special problem of extreme importance, involving knowledge of the factors of resistance and susceptibility, the needs of cultural conditions, and laws of breeding. Notable progress has been made with many crop plants, as oats, cabbage, asparagus, cantaloupes, carnations, flax, melons, and cowpeas.

Hibernation of the parasite has been the subject of much study. In some cases it offers the key to prophylaxis. It is, of course, inseparably linked in many cases with life history studies, and seems also sadly in need of study by those with sound ecological training. Indeed, an ecological study of certain plant parasites, with analysis of the environmental factors and with environments under experimental control, touching also upon seasonal relations, should be very productive. Problems abound on the border fields between mycology, physiology, ecology, and pathology relating to the age relation to disease, to mode of infection, to the climatic and seasonal relations of the parasite, to increase and decrease of susceptibility with changes of environment, to the results of varying the mass of the inoculum, and to change of the virulence of the pathogen with environment.

Epidemiology (to borrow the term from medical usage) is clearly linked with these topics. There is a vast amount of uncorrelated information in the literature concerning the relation between temperature, rainfall, etc., and various diseases, but there is ample

room for a complete "epidemiological" study of any one of many really serious diseases.

A large field involving knowledge of extreme value and demanding ingenuity of experiment is that of pathogen transference. We know a little about transference by insects, but very little about wind and other agents.

Fungicides and their action are in need of more study. It is remarkable if accident has really given us the best fungicides in copper sulphate and lime sulphur. Our knowledge of their action and of their composition can be increased; so, too, the time to apply them and the strength to use. The exact time of application is undoubtedly of much importance; in some diseases, notably apple rust, the variable results are presumably linked with the time relation. Exact knowledge of such relation is needed in many cases. The subject of fungicide injury to fruits or foliage also arises here.

There are many diseases which have been described in a preliminary way, the causes of which are not yet known. Some of these are of great injury, notably the various so-called "mosaics," peach yellows and rosette. Their list is essentially included in the compilation of LANTZ (Ill. Agric. Exp. Sta., Circ. no. 183). It is not too much to hope that some of these will give up their secret under proper attack; some seem to have done so recently; for example, beet curly-top and the crown-gall. The status of others, such as Jonathan spot, tomato blossom-end-rot, tobacco mosaic, and numerous other mosaics, is not so clear. There is here opportunity for good descriptive work that we may know definitely with what conditions we have to deal. When the anatomical, histological relations are definitely recorded, we shall at least be able to classify these various types, and to know, for example, whether in reality the various things that we now call mosaic are of similar or different nature. Abnormal enzyme relations have been invoked to explain some disease conditions, but in general such explanations lack conclusiveness, and certainly lack practical application.

In these remarks I have not aimed at completeness. I have desired rather to indicate the need of intensive, thorough study of the problems before us, and to give a suggestive general view

of the field and of the diversity of research material. It is evident that no one person, either by temperament, inclination, or equipment, is fitted to investigate in all of these fields. The range is broad, and with a veritable wealth of research material, and a survey of the past shows that the well worked subject often is just as productive of results as an apparently much fresher subject. For years the powdery mildews have been introductory subjects in mycology. The group has been thoroughly monographed, collected, listed, years devoted to their biological specialization, treatments devised, etc. The field appeared too thoroughly worked to be promising of large results; yet recent studies have revealed the bud-scale hibernation habit of certain of these, and thus added fundamental knowledge useful in prophylaxis.

Finally, the diseases themselves, not the fungi, need classification. Various classifications have been used, as to cause, as to host, etc., but these do not serve to emphasize relationships of conditions which it is of service to know.

Aside from the non-parasitic diseases, those caused by improper environment of soil, air, light, by abnormal hereditary tendencies, by unknown factors and by predatory animals (including insects), and considering only those known to be caused by parasitic fungi, there are certain groups of conditions which stand out strongly marked as being similar. It is of distinct advantage in studying, in teaching, and in devising and promulgating remedies, to recognize and define these categories. By their very similarity, certain diseases have gravitated together; for example, the vascular diseases, fungous or bacterial diseases, with plugging of the bundles, popularly and very properly call the "wilts."

It is interesting to note that one of the most significant contributions along this line appeared in one of our elementary texts; significant, too, that this contribution should come from one not primarily interested in pathology. COULTER, in his *Elementary studies in botany*, gives us the conception of three general categories of plant diseases: (1) those in which the parasites kill the living cells; as pear blight, spot diseases, downy mildew, potato blight; (2) those in which the parasite does not kill the living cells, but lives in association with them, feeding upon their products; as rust, crown-gall, curl, black knot; (3) those in which the parasites

invade the vessels and live in the sap; as cabbage, cucurbit, and mushroom wilts and wood rot.

I have given some thought to this subject, and, as the only part of this paper which may lay any claim to originality, would present the following suggestions as a step toward a classification of plant diseases caused by fungi, separating them into the following categories:

1. Wilt diseases due to mechanical stoppage of the vascular bundles by parasites. These may be called cases of *embolism;* for example, vascular diseases due to *Ps. campestris*, *B. solanacearum*, and *Acrostilagmus*.

2. Disintegration of the xylem structures; for example, the various wood rots due to *Thelephora*, *Hydnum*, *Poria*, *Polyporus*, *Fomes*, *Trametes*, *Dedalea*, *Schizophyllum*, etc.

3. Diseases due to parasites wholly contained within the living protoplasm of the host cell. This is the strictest type of parasitism, and may appropriately be known as *endocellular parasitism;* for example, diseases due to *Synchytrium*.

4. Diseases due to parasites which draw their nutriment from living cells by haustoria, which may be called *endocellular haustorial parasitism;* for example, diseases due to *Phyllactinia*, *Peronospora*, *Albugo*, and *Plasmopara*. In this group the conspicuous feature is the relatively large development of the haustorial surface as compared with the remainder of the internal mycelium.

5. Diseases in which the live epidermal cells only are directly parasitized. These may be called cases of *epidermitis*, for example, diseases due to the Erysiphales (exclusive of *Phyllactinia*), *Meliola*.

6. Diseases in which the parasite grows between the living host cells. Haustoria may be present, but if so they are not prominent, and the apparently dominant part of the absorptive system is the intercellular mycelium. This may be called *intercellular mycosis;* for example, rusts, Exoascales, Exobasidiales, and *Cephaleurus*.

7. Diseases in which the host tissue is displaced or replaced by fungous masses. This may be called *mycosclerosis;* for example, diseases caused by *Claviceps*, *Phyllachora*, *Rhytisma*, and the smuts.

8. Diseases of the type produced by *Pseudomonas tumefaciens*, which may be called *tumor*.

9. Diseases in which the dominant feature is death of the host cells before they are actually invaded by the parasite. To these may be applied the term *necrosis*. Subdivision may be made on the basis of the part involved, as:

9*a*. *Cortical necrosis*, in which the cortex chiefly is involved; for example, cankers caused by *Sphaeropsis*, *B. amylovorus*, and *Endothia*.

9*b*. *Parenchymal necrosis*, in which chiefly the parenchyma is affected, including the greater number of the soft rots; for example, soft rots caused by *B. carotovorus*, *Rhizopus*, *Penicillium*, *Phythiacysitis*, *Rhizoctonia*, *Pythium*, *Phytophthora*, *Sclerotinia*, *Botrytis*, *Colletotrichum*, and *Gleosporium*.

9*c*. *Macular necrosis*, in which necrosis is limited to spots, chiefly occurring on leaves. This is divided into (1) macular necrosis with abscission (the "shothole" diseases caused, for example, by *Cylindrosporium* and *Marssonia*); (2) macular necrosis without abscission (chiefly the leaf spots, caused, for example, by *Pseudopeziza*, *Entomosporium*, *Macrosporium*, *Lophioderma*, *Guignardia* [*Phyllosticta*], *Ascochyta*, *Ramularia*, *Septoria*, *Diplodia*, *Cercospora*, *Colletotrichum*, *Gleosporium*, *Fusicladium*, *Cladosporium*, and *Alternaria*.

The following synopsis may make these categories and their interrelations clear.

I. The parasite living in the sap or in cavities or parts devoid of living protoplasm: (1) embolism; (2) wood rots.

II. The parasite for the major part of its life drawing its nutriment from host cells that are still living: (3) endocellular parasitism; (4) endocellular haustorial parasitism; (5) epidermitis; (6) intercellular mycosis; (7) mycosclerosis; (8) tumor.

III. The parasite living within host cells or tissues which have recently been killed or partially disorganized by it: (9) necrosis; (9*a*) cortical necrosis; (9*b*) parenchymal necrosis; (9*c*) macular necrosis; (9*c'*) macular necrosis with abscission; (9*c''*) macular necrosis without abscission.

There is an apparent omission of hypertrophy and hyperplasia, but I regard these two manifestations as symptoms rather than as definite diseases.

UNIVERSITY OF ILLINOIS

FLOWERS AND INSECTS. XX

EVOLUTION OF ENTOMOPHILOUS FLOWERS

CHARLES ROBERTSON

In his *Fertilisation of flowers* (pp. 594, 595) MÜLLER arrives at the following conclusions with regard to the development of flowers:

The transition from wind fertilization to insect fertilization, and the first traces of adaptation to insects, could only be due to the influence of quite short-lipped insects with feebly developed color-sense. The most primitive flowers are therefore for the most part (except, for instance, *Salix*) simple, widely open, regular, devoid of honey or with their honey unconcealed and easily accessible, and white or yellow in color (for example, most Umbelliferae and Alsineae, many Ranunculaceae and Rosaceae).

Gradually, from the miscellaneous lot of flower-visiting insects, all much alike in their tastes, there arose others more skilful and intelligent, with longer tongues and acuter color-sense; and they gradually caused the production of flowers with more varied colors, honey invisible to or beyond the reach of the less intelligent short-tongued guests, and various contrivances for lodging, protecting, and pointing out the honey.

The Ichneumonidae at first surpassed all other visitors in observation and discernment, and they were thus able to produce inconspicuous flowers which escaped the notice of other visitors. On the appearance of sand wasps and bees these inconspicuous flowers were banished by competition to the less frequented localities (for example, *Listera* to shady woods).

The sand wasps (Sphegidae) apparently took the place to a great extent of the ichneumons, and produced flowers where organs had to be thrust apart (Papilionaceae), or where a narrow cavity had to be entered (Labiatae), or where some other action similar to the act of digging had to be performed. Subsequently bees seem to have entered on joint possession of most of these flowers, and to have added special adaptations of their own.

The true wasps (Vespidae) could establish themselves by the fear of their sting (and of their jaws) in sole possession of certain flowers with wide open mouths and abundant honey. These they developed further in relation to their wants (*Scrophularia*, *Symphoricarpos*, *Epipactis latifolia*, *Lonicera alpigena*); but where wasps are scarce the flowers are utilized by other insects.

Bees (Apidae), as the most skilful and diligent visitors, have played the chief part in the evolution of flowers; we owe to them the most numerous, most varied, and most specialized forms.

Whether the primitive flowers were pollinated by wind or by insects is uncertain. The forms of flowers which preceded the angiosperms were probably entomophilous. The carpels closed over the ovules to form an ovary and the stigma was developed to receive the pollen. The stigma and closed ovary are regarded as entomophilous characters and as having been developed after the visits of insects were established. The origin and development of entomophilous flowers, no doubt, were connected with the origin and specialization of the bees, Hymenoptera which adopted the habit of provisioning their nests with nectar and pollen. Along with the acquisition of this habit, the bees developed a coat of feathery hairs to which the pollen might cling, these hairs on certain parts of their bodies, as the hind legs and the ventral surface of the abdomen, being greatly modified to form a special pollen-carrying apparatus. Thus the pollen became absolutely essential in the economy of the bees. To the flowers, on the other hand, the bees became important visitors, because they had to resort to flowers frequently and because they were provided with a coat specially fitted to retain the pollen, and at the same time exerted themselves to get the coat as full of pollen as possible.

Bees, as we know them, visit flowers both for nectar and for pollen, but it is possible that the primitive bees visited flowers only for pollen and that the secretion of nectar came after.

The view has been expressed[1] that the ordinary short-tongued bees can collect only viscid pollen, and that therefore they could have begun to use pollen to provision their nests only after pollen had become sticky in adaptation to insect pollination. Species of *Chloralictus* collect the dry pollen of grasses and of *Plantago*, however, and ordinary bees collect from a considerable number of flowers pollen which is so dry that it pours out as soon as it is released from the anthers. So bees may have commenced to collect pollen when only dry pollen existed. The fact that bees are the most highly specialized of Hymenoptera, and the latest developed, does not prove, and does not seem to establish a reasonable presumption, that any considerable evolution of entomophilous flowers preceded their advent.

[1] ROBERTSON, CHARLES, Flowers and insects. XIX. BOT. GAZ. **28**: 39. 1899.

Putting speculation aside, the further consideration of this subject will be limited to the structure and affinities of the flowers, and the behavior of the insects which we know. Social flowers are those which are so closely approximated that the visitors may readily pass from one to another without taking wing or climbing. They are usually found in heads, spikes, or close umbels. The simplest flowers which we know are non-social flowers of class *AB*, flowers with partly concealed nectar. Insect visits to them show:

	Species	Bees	Diptera	Other Hymenoptera	Lepidoptera	Coleoptera Hemiptera	Total
Class *AB*	41	56.8	31.2	4.7	4.8	2.3	866
Visits	14	43.7	32.8	19.5	2.7	1.2	405
Individuals	0	70.2	20.4	8.0	0.7	0.5	2438

These are evidently bee flowers, although they are not exclusively visited by bees. No insects except bees prefer flowers of this kind. There are no non-social flowers of class *AB* which are adapted to miscellaneous insects or to particular kinds of visitors except bees. On 14 species of class *AB* bees showed 43.7 per cent of the visits and 70.2 per cent of the individuals. Of course it is possible that the primitive non-social flowers of class *AB* were visited by a miscellaneous set of the least specialized anthophilous insects. If so, the short-tongued bees must have tended early to monopolize them, while the other insects paid more attention to the forms which became social.

Observations of 221 visits to 17 non-social flowers of class *A*, flowers with exposed nectar, show: bees 33.4, Diptera 45.7, other Hymenoptera 14.4, Coleoptera and Hemiptera 6.3. Here the Diptera predominate, and the group is rather miscellaneous. Some of the group are distinct fly-flowers (*Asimina triloba*); some are quite simple (*Asimina*, *Myosurus*, and *Caulophyllum*). The dark color and pendulous position of *Asimina* are hardly typical, and *Myosurus* and *Caulophyllum* have peculiar petals. None of these are simple like ordinary non-social flowers of class *AB*. Most non-social *A* have epigynous nectaries (*Hypoxis*, *Circaea*,

Galium). A characteristic flower is *Circaea lutetiana*. Its visitors are:

	Bees	Diptera	Total
Species	81.8	18.1	11
Individuals	94.0	5.9	84

Class *A* is a poor place to look for simple flowers. The majority are social and have epigynous nectaries, both forms of specialization. Except class *B*,[1] this class is the only one in which the majority of the species are social. The visits to 23 social *A* are as follows: bees 21.9, Diptera 38.3, other Hymenoptera 27.3, Lepidoptera 2.6, Coleoptera and Hemiptera 9.6, making a total of 2335.

Table I, based on 10,041 visits, shows the percentages of visits of all classes to flowers adapted to short-tongued insects, usually small flowers with nectar exposed, partly or wholly concealed, but never deep seated.

TABLE I

	Bees	Diptera	Lepidoptera	Coleoptera	Hemiptera	Lower Hymenoptera
PERCENTAGE OF VISITS						
To non-social flowers	18.1	12.4	11.2	8.6	6.1	4.1
To social flowers	41.8	73.5	34.8	83.3	78.7	84.9
PERCENTAGE OF TOTAL VISITS						
To non-social flowers	59.5	25.3	7.2	2.0	0.3	5.3
To social flowers	31.0	33.8	5.0	4.4	1.0	24.4

Of the visits of bees, 18.1 per cent are to non-social small flowers, and these form 59.5 per cent of the total insect visits to such flowers. Sixteen non-social small flowers, on which the individual insects were taken as they came and counted, showed 335 visits and 1520 individuals. The percentage of bee visits was 59.1, but of bee individuals 74.2, showing that bees are more important than the percentage of visits indicates.

The relations of bees and other insects to non-social and social flowers in general (based upon 13,942 visits of 1287 insects to 437 flowers) are shown in table II.

TABLE II

	Lower Hymenoptera	Hemiptera	Coleoptera	Diptera	Lepidoptera	Bees	All Except Bees	Total
PERCENTAGE OF VISITS								
To non-social flowers	4.4	6.1	9.3	13.6	22.6	32.1	11.9	20.7
To social flowers	95.5	93.8	90.6	86.3	77.3	67.8	88.0	79.2
PERCENTAGE OF TOTAL VISITS								
To non-social flowers	3.6	0.2	1.4	17.8	9.3	67.4	32.5	99.9
To social flowers	20.4	0.9	3.5	29.4	8.3	37.2	62.7	99.9

Of the total visits of bees, 32.1 per cent are to non-social flowers, and these form 67.4 per cent of the total insect visits to such flowers. Of the total visits of other insects to non-social flowers, the percentage is 4.4 for lower Hymenoptera, 6.1 for Hemiptera, 9.3 for Coleoptera, 13.6 for Diptera, and 22.6 for Lepidoptera; or a general percentage of 11.9. Since bees make over two-thirds of the insect visits to non-social flowers, it is evident that they have been chiefly instrumental in the origination of such flowers.

Of the total visits of bees, 67.8 per cent are to social flowers, so that bees show a strong preference for these flowers also, although not as strong a preference as the Lepidoptera with 77.3, the Diptera with 86.3, the Coleoptera with 90.6, the Hemiptera with 93.8, and the lower Hymenoptera with 95.5 per cent.

One might suppose, with MÜLLER, that the non-aculeate Hymenoptera have had an influence in the development of some primitive flowers, and that these flowers were further modified by the aculeate Hymenoptera, and finally became highly specialized in connection with the development and specialization of the bees. When, however, we look for such flowers, we find only the so-called ichneumon flowers, *Listera ovata* and *Chamaerorchis alpina*, belonging to the most highly specialized of monocotyledons. In the

case of the Ichneumonidae only 2.5 per cent of the visits are to non-social flowers.

The only flowers supposed to have been modified by the Vespidae are the so-called wasp flowers, *Epipactis latifolia* (Orchidaceae) belonging to the most highly specialized group of non-social monocotyledons, *Scrophularia nodosa* (Scrophulariaceae) belonging to a distinctly melittophilous family, *Lonicera alpigena* belonging to a melittophilous genus, and *Symphoricarpos racemosus* belonging to the epigynous Caprifoliaceae. None of these belong to primitive forms of flowers which might have preceded the advent of the bees. Only 8.7 per cent of the visits of Vespidae are to non-social flowers.

With the exception of *Symphoricarpos*, all of the flowers mentioned by MÜLLER as having been modified in adaptation to the lower Hymenoptera are zygomorphous: Orchidaceae, Papilionaceae, Labiatae, *Scrophularia*, and *Lonicera alpigena*. Zygomorphous flowers, except such forms as *Aristolochia*, with *siphonate* zygomorphy, and the outer flowers of the umbels of *Heracleum*, with *radiate* zygomorphy, are typically non-social and adapted to bees which visit each flower separately. They have a landing either above or below the stamens and pistils and usually dust the visitor on the lower or upper side. It is fairly inconceivable that zygomorphy should have originated in crowded inflorescences where the flowers might be approached from any side. Excluding such flowers as *Heracleum*, *Aristolochia; Amorpha*, *Petalostemon*, and *Melilotus* in Papilionaceae; and *Pycnanthemum*, *Lycopus*, and *Mentha* in Labiatae, 100 zygomorphous flowers show: bees 74.3, Diptera 8.5, other Hymenoptera 9.1, Lepidoptera 7.1, Coleoptera and Hemiptera 0.7, making a total of 1117 visits.

Visits to the Papilionaceae show:

	Bees	Diptera	Other Hymenoptera	Lepidoptera	Coleoptera, Hemiptera	Total
Non-social (24)	97.5	1.6	0.8	0.0	0.0	123
Social (9)	56.1	16.3	23.0	1.5	2.9	447
Total (33)	65.0	13.1	18.2	1.2	2.2	570
Amorpha, etc. (4)	45.3	18.5	29.4	2.3	4.3	302

When the lower Hymenoptera together make only 0.8 per cent of the visits to the non-social Papilionaceae, it is evident that they have had little to do with the evolution of the Papilionaceae, even if they were instrumental in their origin. To support the latter condition it would be necessary to show that the non-social forms were developed from the social forms.

Visits to Labiatae show:

	Bees	Diptera	Other Hymenoptera	Lepidoptera	Coleoptera, Hemiptera	Total
Non-social (13)	83.1	5.8	0.8	10.0	0.0	119
Social (12)	39.5	20.8	24.6	12.7	2.2	897
Total (25)	44.6	19.0	21.8	12.4	1.9	1016
Lycopus, etc. (5)	29.6	24.6	34.5	7.8	3.2	576

When the lower Hymenoptera show 24.6 per cent of the visits to social Labiatae and only 0.8 per cent to non-social Labiatae, it is hard to connect them with the origin of the Labiatae unless we suppose that the non-social developed from the social. MÜLLER (*Fertilisation of flowers*, p. 471) says: "DELPINO considers *Mentha* and *Coleus* degraded forms of the labiate type; he, however, gives no reason for thinking them to be such, and not rather less specialized forms, differing less from the common ancestors of the Labiatae." If there are non-social zygomorphous wasp flowers or ichneumon flowers, no doubt they should be regarded as modified from bee flowers.

The view held here, that the early flowers were non-social and were modified in connection with the visits of bees, and that the flowers mainly visited by other insects are later, is supported by what is known of the behavior of insects and by inferences from the affinities of the flowers. Of course, if it can be shown that the primitive flowers were social and that the non-social flowers were developed from them, this view will have to be abandoned for that of MÜLLER.

Of the total visits of the lower insects, 88.0 per cent are to social flowers, and of the total insect visits to social flowers the lower insects make 62.7 per cent. Now the flowers which these insects

prefer are not the simple ones, but the majority are social and have epigynous nectaries.

The original or normal bees are polylectic. They have a general relation to the flora and more special relations to certain flower classes. From these have originated the oligolectic bees and inquilines. The oligoleges collect pollen exclusively from flowers belonging to particular natural groups. They do not prefer flower classes except in so far as their particular flowers happen to belong to those classes. The inquiline bees live in the nests and at the expense of the other bees. They get only nectar from the flowers which happen to be the most convenient and easiest for them to visit. The importance to the flora of these 3 sets of bees is partly indicated in table III.

TABLE III

	Species	Visits	Average
Normal, polyleges	132	4448	33.6
Inquilines	72	781	10.8
Oligoleges	83	668	8.0
Prosopis	9	166	18.4
Total	296	6063	20.4

In a considerable number of polyleges the flight of the males is quite different from that of the females. The males do not make half as many visits as the females, and the flowers which they visit are so different that their visits to flowers should be considered separately. Table IV shows the differences.

TABLE IV

	Females (♂ excluded)			Males		
	Number	Visits	Average	Number	Visits	Average
Large polyleges	50	1239	24.6	50	806	16.1
Small polyleges	72	2098	29.1	64	722	11.2
Total	122	3337	27.3	114	1528	13.3

The groups of visitors preferring certain classes of non-social flowers are separated as shown in table V. Usually large flowers

with deep seated nectar are referred to as *Ma*, usually small flowers with nectar not deep seated are referred to as *Mi*, while *Pol* indicates the extreme social forms.

TABLE V

	Non-social			Social				Total
	Ma	Mi	Total	Ma	Mi	Pol	Total	
Flora	30.2	24.2	54.4	18.7	21.5	5.2	45.5	437
Small bees, polyleges ♀	5.0	30.4	35.5	11.7	43.6	9.0	64.4	2098
Large bees, polyleges ♀	35.9	13.4	49.4	30.9	15.8	3.7	50.5	1239
Sphingidae	54.5	0.0	54.5	40.9	4.5	0.0	45.4	22
Humming-bird (*Trochilus*)	82.7	3.4	62.2	13.7	0.0	0.0	13.7	29

The females of the short-tongued polylectic bees form the only group of insects preferring non-social *Mi*. They are credited with the origin of such flowers. The females of the long-tongued polylectic bees, the Sphingidae, and *Trochilus* are the only groups preferring non-social *Ma*. This is the largest flower class, originally modified by long-tongued bees. The Sphingidae and *Trochilus* prefer such flowers and in some cases have entirely appropriated them.

As flowers have become social they have been preferred in the order shown in table VI.

TABLE VI

Groups of insects	Non-social	Social
Large bees, polyleges ♂	37.8	62.1
Lepidoptera (ex. Sphingidae)	22.0	77.9
Large bees, inquilines	20.6	79.3
Small bees, polyleges ♂	19.5	80.4
Prosopis	13.8	86.1
Diptera	13.6	86.3
Large bees, oligoleges	13.4	86.5
Small bees, inquilines ♀	12.7	87.2
Small bees, oligoleges	11.3	88.6
Coleoptera	9.3	90.6
Hemiptera	6.1	93.8
Lower Hymenoptera	4.4	95.5
Small bees, inquilines ♂	4.0	96.0

Some female bees on their pollen visits show a preference for social flowers. Eighty-five species of long-tongued bees, with

806 pollen visits, and 1155 nectar visits of females and workers, show the following percentages of visits to social flowers with exposed pollen: for nectar 41.1; for pollen 49.2. Compared with the visits of the females for nectar, the females when collecting pollen make 8.1 per cent more visits to social flowers.

There are some large social inflorescences composed of flowers with exposed or only slightly concealed nectar. Long-tongued bees practically avoid them on their nectar visits, but often visit them for pollen. Such are *Cornus*, *Hydrangea*, and *Viburnum*. *Vitis*, with exposed nectar, seems to be an important source of pollen for female bumblebees. The aggregation of flowers in social clusters has been interpreted as an adaptation for gitonogamy, but it occurs about as often in cases where gitonogamy is impossible.

Finally, the evolution of entomophilous flowers is held to have proceeded in the following manner. The primitive flowers were non-social flowers of class *AB*, with partly concealed nectar, adapted to short-tongued bees. These have produced flowers with exposed nectar more favorable to flies, and flowers with more concealed nectar still more favorable to bees. A few have become adapted to flesh flies (*Asimina*), and others to minute flies (*Aristolochia*).

The non-social small bee flowers have produced social forms still favoring small bees, but admitting other short-tongued insects. These finally pass into the extreme social forms which have become modified to suit miscellaneous short-tongued insects.

The non-social small bee flowers have been modified further and developed into non-social long-tongued bee flowers. Some of these have been appropriated by birds and others by Sphingidae, and perhaps still others by butterflies.

The non-social long-tongued bee flowers have also been modified into social forms attracting Lepidoptera and long-tongued Diptera. These are still considered as bee flowers, but some of them may more properly be regarded as adapted to miscellaneous long-tongued insects. The social long-tongued bee flowers also pass into social short-tongued bee flowers, and finally into social flowers adapted to miscellaneous short-tongued insects.

CARLINVILLE, ILL.

DOES THE TEMPERATURE COEFFICIENT OF PERMEABILITY INDICATE THAT IT IS CHEMICAL IN NATURE?

W. J. V. OSTERHOUT

In a recent paper STILES and JORGENSEN[1] state that the absorption of hydrogen ions by tissues of the potato has the temperature coefficient of a chemical reaction (2.18–2.22). They apparently reach the conclusion that "the substance with which the acid reacts" is "presumably the plasma membrane or some part of it," and that the facts suggest the view "held by PAULI and SZÜCS, who regard the entrance of ions into the cell as due to the reversibility of such a reaction between ions and the plasma membrane." These statements, together with the title of their paper, "The effect of temperature on the permeability of plant cells to the hydrogen ion," indicate that they regard the temperature coefficient found by them as the temperature coefficient of permeability to hydrogen ions.

This view, if well founded, is of considerable interest, as it indicates that permeability is chemical[2] rather than physical in nature, since (unless vapor tension is a determining factor) no physical processes are apt to be involved in this case which have a temperature coefficient as high as 2.[3] In view of this the statements of STILES and JORGENSEN deserve careful examination.

It should be noted that the only criterion of permeability employed by them was absorption from a solution. Their method consisted in placing slices of potato in a solution of HCl and

[1] Ann. Botany **29**:611. 1915.

[2] BROWN and WORLEY (Proc. Roy. Soc. London B. **85**:546. 1912) have shown that the temperature coefficient of absorption of water by seeds is 2, but it is not clear whether this applies to imbibition (or other processes) taking place inside the cells, or to the permeability of the protoplasm. If it is really the coefficient of permeability to water, it is by no means necessary to extend this conception to permeability to substances other than water.

[3] Cf. KANITZ, A., Temperatur und Lebensvorgänge. Berlin. 1915 (p. 105).

determining the loss of hydrogen ions from the solution by means of a hydrogen electrode.

It may be observed in this connection that the absorption of dissolved substances by living cells has been employed extensively as a criterion of permeability. The amount of absorption is usually determined by analysis (of the solution or of the tissue) before and after the organism is placed in the solution. A more convenient method, which suffices in some cases, is to determine the conductivity of the solution. Nephelometry is also useful. Such methods may also be used to determine the excretion of substances by the organism.

The results obtained by these methods have been so largely misinterpreted that there is widespread confusion in regard to their significance. This confusion is due in part to uncritical technique and in part to overlooking some of the many variables involved in such experiments; but the principal difficulty lies in confusing permeability with absorption.

The nature of this difficulty is evident from the following illustration. Suppose a glass tube closed at one end by a membrane in contact with a solution to which it is freely permeable. The solution will pass through the membrane into the tube until equilibrium is established. If, however, we place in the tube something which precipitates the dissolved substance, more of the latter will diffuse in, and this will go on as long as the precipitation continues. It is not even necessary that the precipitation should occur, since the result can be obtained by causing the dissolved substance to unite with something within the tube so as to form a compound which cannot pass out through the membrane.[4] The dissolved substance will then continue to pass into the tube.

It is evident that the permeability of the membrane remains the same whether precipitation or other chemical action occurs or not. But while the permeability remains the same, the amount of adsorption will vary enormously.

This may be observed with the living cell. When a cell is placed in a dye which is precipitated within the cell (giving a visible precipitate), the absorption of the dye goes on as long as

[4] Cf. LOEB, J., Dynamics of living matter. 1906 (p. 72).

the precipitate continues to form, while in the case of a dye which is not precipitated (and which does not form a compound incapable of passing out), the absorption ceases as soon as the concentration within the cell equals that of the solution.

It is evident, therefore, that the temperature coefficient observed by STILES and JORGENSEN may be that of a chemical process[5] involving the union of hydrogen ions with some constituent of the cell other than the plasma membrane (or other surface), in which case it would have no bearing upon the problem of the nature of permeability.[6]

Some time ago the writer sought to throw some light on this problem in ascertaining the temperature coefficient of permeability by a method which is free from the objections just discussed. By this method[7] the electrical conductivity of living tissue was determined in such a way that it may be regarded as a measure of the permeability of the protoplasm.

In these experiments a series of disks of *Laminaria* were packed together (like a roll of coins) so as to form a solid cylinder about 2 inches in length. The electrical conductivity was then measured at various temperatures. The temperature coefficient obtained in in this way was 1.33. The tissue was subsequently killed, whereby the conductivity was increased to practically that of sea water. The temperature coefficient of the dead tissue proved to be 1.26, which is practically the same as that of sea water.

If most of the resistance were due to apparatus, cell walls (intercellular substance), and sea water, and these had low temperature coefficients (for example, 1.26), that part of the resistance which is due to living protoplasm might have a high temperature coefficient (for example, 2) without much raising the temperature coefficient of the total resistance. The resistance of the apparatus (and the sea water contained in it) was determined at each temperature and subtracted from the total (giving what is called the

[5] Absorption may also play a part in this connection.

[6] Cf. Biochem. Zeitschr. **67**:272. 1914.

[7] Even if the hydrogen ion unites "with the plasma membrane or some part of it," the temperature coefficient of this process would not necessarily be the temperature coefficient of permeability.

net resistance), so that we need consider only the resistance of the protoplasm, of the cell walls imbibed with sea water, and of the capillary films of sea water between the disks.

When the net resistance is 1200 ohms, we find that on killing the tissue the resistance drops to about 100 ohms. Since this represents the resistance of the cell walls and of the sea water plus the resistance of the dead protoplasm, it is evident that the resistance of the cell walls and of the sea water together must be less than 100 ohms. So far as can be judged from microscopic measurements, the fraction of the cross-section of the conducting column occupied by the cell walls is less than half, and it is probable that when the net resistance is 1000 ohms, not more than 50 ohms are due to cell walls and to the sea water adhering to the tissues.[8]

It is evident, therefore, that if the resistance of the living protoplasm had a temperature coefficient of 2, the temperature coefficient of the total resistance would be only a little less than 2.

We may conclude, therefore, that the temperature coefficient of permeability is not far above 1.33. This indicates that permeability is not chemical in nature, although it is not absolute proof, as some chemical reactions have low temperature coefficients.

It would seem, therefore, that we cannot accept the idea that permeability is chemical in nature without much more conclusive evidence than we possess at present.

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[8] The problem is complicated by the arrangement of the protoplasmic masses.

BRIEFER ARTICLES

A METHOD FOR PRODUCING CONDUCTIVITY WATER SUITABLE FOR WATER CULTURE EXPERIMENTS[1]

(WITH ONE FIGURE)

An examination of one of the most recent types of water stills has convinced the author that there is abundant opportunity for the presence of copper and other metals in the distillate. The boiler is made of copper, not completely covered by tin; consequently, a coating of copper carbonate forms which may be conducted over in the spray during rapid distillation. Although the condenser itself may be of block tin, there is ample opportunity for contact of the distillate with brass connections.

On account of the reputed great physiological activity of copper, it was deemed advisable to use water free from the suspicion of contamination. This led the author to devise a method for producing water of high resistance in sufficiently large quantity for water culture experiments. The apparatus here described has been used for some time and has been found entirely satisfactory.

The level of water in a tubulatured retort is regulated automatically by a siphon (1) which discharges to a constant level in receiver (3). The end of the water tube (2) of the regulator is so adjusted that air can rise into the reservoir above only when the level in the retort is lowered slightly by distillation. Then the vacuum pressure is relieved slightly so as to allow water to flow into the siphon.

It was found necessary to provide the water seal siphon with an upright tube to prevent the stoppage of the siphon by gases expelled from the water when heated.

The constant level of the water in the retort offers the advantage of greater space above the boiling liquid to minimize the danger of spray being carried over. A pledget of glass wool is placed in the bent neck of the retort to remove spray and return it to the retort. Condensation of the distillate is affected by a glass water jacket fitted to the neck of the retort by rubber stoppers.

[1] Published by permission of the Secretary of Agriculture.

The system can be isolated almost entirely from the atmosphere by the protecting tubes and the water seals as shown in the diagram. The principal advantages of the apparatus are that it requires little attention

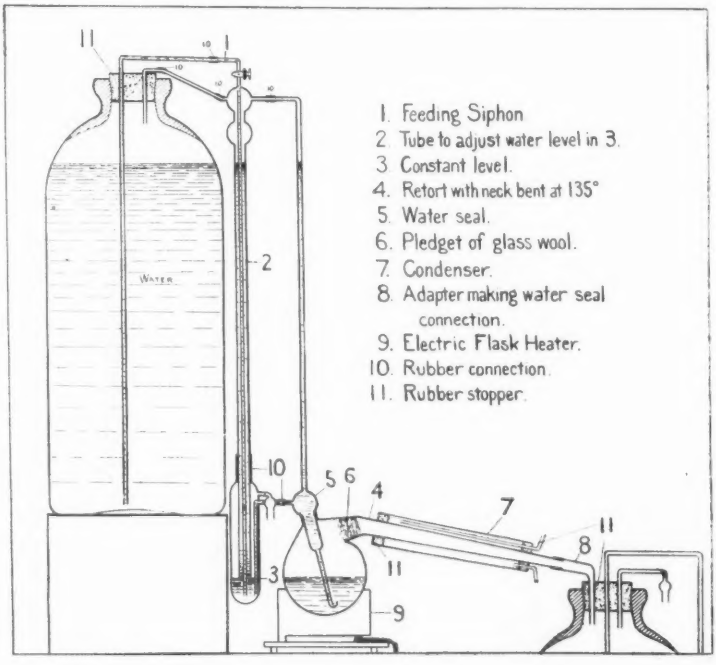
1. Feeding Siphon
2. Tube to adjust water level in 3.
3. Constant level.
4. Retort with neck bent at 135°
5. Water seal.
6. Pledget of glass wool.
7. Condenser.
8. Adapter making water seal connection.
9. Electric Flask Heater.
10. Rubber connection.
11. Rubber stopper.
WATER

FIG. 1

beyond occasional cleaning, and can be relied upon to produce a constant supply of high resistance water containing only materials dissolved from slightly soluble glass.—RODNEY B. HARVEY, *U.S. Department of Agriculture, Washington, D.C.*

CURRENT LITERATURE

BOOK REVIEWS

Two new college texts

The two recent texts by GANONG and GAGER agree in the modern spirit evident in each, but they differ distinctly in the selection and arrangement of material. In GANONG's text[1] the arrangement of topics is primarily morphological. This arrangement will undoubtedly prove here, as it has in many an earlier text, its peculiar fitness for an introductory textbook, because of simplicity and its ready intelligibility to the beginner, who already knows the primary organs of the plant by sight and by name. In the 6 chapters of the body of the book the discussion in succession of leaf, stem, root, flower, fruit, and seed is carried out in each case with that close interrelating of structure and function which is essential to the clear comprehension of the relative significance and of the intimate interdependence of these two phases of plant organization. Each of these chapters includes a section on the economics and cultivation of the structure under consideration. The chapter on the flower contains concise but clear discussions of the significance of sex, of heredity, of evolution, and of plant breeding, while the following chapter has a brief section on plant diseases. Ecology and paleobotany are not discussed in this part.

The 274 illustrations include a considerable number of original ones, of which figs. 85 and 162 are excellent examples, and there are many less frequently copied ones from various standard works. It is not clear to the reviewer, however, that such synthetic diagrams as those of the leaf, stem, and root (figs. 11, 105, 166) are really needed by the average student. They do suggest, it is true, certain more important features of the structure and work of each organ, but because of the omission, for the sake of simplicity, of some other essential structures they are liable to be misleading. Properly selected, accurate drawings would avoid this objection and still be entirely intelligible. The reviewer is inclined to question also whether the substitution of drawings of models showing leaf arrangement, for figures of the stems and leaves themselves, is really necessary as an aid to the imagination of the ordinary college student. The second part, entitled "The kinds and relationships of plants," is expected to be ready during 1917.

[1] GANONG, WILLIAM F., A textbook of botany for colleges. Part I. The structures and functions of plants. 8vo. pp. xi+401. New York: Macmillan. 1916.

GAGER'S text[2] is evidently intended, like GANONG'S, as a guide for an introductory, cultural course for college students, which shall at the same time serve as a foundational one for students who are to pursue the subject further. The arrangement of topics, however, differs in being professedly physiological, at least in Part II, which corresponds most nearly to the body of GANONG'S book. Part I ("Introduction") deals with the organs of the cormophyte and the structure of the cell. Part II ("The vegetative functions of plants") includes chapters on the loss of water, absorption of water, the path of liquids in the plant, nutrition, fermentation, respiration, growth, and adjustment to surroundings.

Chapter IV, under the title "Loss of water," which does not very adequately forewarn the reader of the nature of its contents, discusses some of the essential facts of the gross morphology, histology, and physiology of the leaf. Chapter V ("Absorption of water") treats of the absorptive function of the soil root, but other functions and the structure of the root, aside from that of the root hairs, are not considered here, nor could anything but the briefest mention of them be found elsewhere in the book. Certain important features of the structure and activity of the stem also are either not referred to at all, or are barely mentioned. Thus, such tissues as bark, phellogen, cork, sieve tubes, etc., are not mentioned in the index, or more than incidentally referred to in the text. Secondary thickening, types of branching, the various habits assumed by the stem, the structure of buds, etc., are not given space for any real discussion or explanation. These omissions are apparently part of the plan of the book and are interesting as showing the author's estimate of the relative importance of these topics among the large number from which selection must be made.

The 26 chapters of Part III ("Structure and life histories") include discussions of the life histories of a considerable number of types, especially of the mosses, ferns, and flowering plants. The fern is made the primary type in these discussions of life cycles, and the whole series is rather copiously illustrated. Important and interestingly written chapters in this part are those dealing with the problem of sex in plants, the economic importance of fungi, evolution, Darwinism, experimental evolution, heredity, and paleobotany. The treatment of these themes, with the series of accompanying portraits of some of the great naturalists, serves to suggest something of the history of certain important botanical theories.

The book is abundantly illustrated with 434 figures, a good share of which are original drawings or halftones. While the appearance, for example, of such illustrations as figs. 127, 198, 263, and 286 is to be welcomed, the same cannot justly be said of some others. The use of such illustrations as figs. 37, 142, 151, 160, 165, or 180 would seem scarcely justified on grounds of mere novelty,

[2] GAGER, C. STUART, Fundamentals of botany. 8vo. pp. xvi+640. Philadelphia: Blakiston's Sons. 1916.

when much clearer ones of the same objects are already available.—D. S. JOHNSON.

MINOR NOTICES

North American flora.—The third part of Vol. 34 continues the presentation of Carduales by RYDBERG,[3] including the completion of the Tageteae and the Anthemideae. In the Tageteae 22 genera are recognized, the last 5 being presented in this part. Of these the large genera are *Pectis* with 71 species (11 new) and *Porophyllum* with 42 species (10 new). A new genus (*Hydropectis*) is described, based on *Pectis aquatica* Wats.

The recognized genera of Anthemideae number 21, a considerable number of them being segregated from more familiar genera. The largest genus is *Artemisia* with 120 species (29 new), followed by *Achillea* with 24 species (6 new). The other genera are represented by comparatively few species. Three new genera are described as follows: *Vesicarpa*, based on *Artemisia potentilloides* Gray; *Chamartemisia*, based on *Tanacetum compactum* Hall; *Artemisiastrum*, based on *Artemisia Palmeri* Gray.—J. M. C.

The theory of evolution.—SCOTT[4] has made an excellent restatement of the evidences of organic evolution. The somewhat hackneyed subject is enlivened by a forcible and very readable presentation. The book is the result of the organization of 6 lectures (the Westbrook Lectures for 1914). In addition to the evidences from classification, comparative anatomy, embryology, paleontology, and geographical distribution, the author presents evidence derived from domestication, from blood tests, and from experiment.

The opening chapter gives a brief historical review of theories of evolution and a concise statement of the present status of the question. I have seen no better presentation of this body of data for both biologist and general reader than that given in this little book. My only criticism is that it is insufficiently illustrated, although the few illustrations used are well chosen—H. H. NEWMAN.

A moss flora.—GROUT[5] has published a very convenient list of the moss flora of all counties of New York and New Jersey adjacent to New York City. The moss flora of this area has probably been explored more thoroughly than that of any other region of the United States. Numerous keys make the recognition of genera and species relatively easy, and the excellent photographic plates illustrate the genera. Such a publication should stimulate the study of a very interesting flora, for, as the author remarks, "in and around New York

[3] RYDBERG, PER AXEL, North American Flora **34**:part 3. pp. 181–288. Carduales: Carduaceae (Tageteae, Anthemideae). New York Botanic Garden. 1916.

[4] SCOTT, W. B., The theory of evolution. 8vo. pp. vii+183. New York: Macmillan. 1917.

[5] GROUT, A. J., The moss flora of New York City and vicinity. 8vo. pp. 120, *pls. 12*. New Dorp (N.Y.): published by the author. 1916.

City the moss flora of the north and of the south meet and mingle, and the number of species occurring is large, varied, and interesting."—J. M. C.

NOTES FOR STUDENTS

Transpiration studies.—Among several recent papers dealing with various phases of the study of transpiration, a prominent place should be given to one by LIVINGSTON and SHREVE[6] upon improvements in the use of the cobalt chloride paper method. An improved paper slip has been designed which combines two permanent color standards and an area of carefully prepared cobalt chloride paper. The determinations of the end points are made more definite, therefore, and the probability of error is much reduced. An improved device for furnishing a standard water surface is described also. The temperature relations of the rate of color change in the hygrometric paper and its permanent standardization is discussed also. These improvements will greatly advance the method of study which has already been proved valuable.

Another modification of methods of study is seen in DARWIN's[7] investigation of the relation of transpiration to relative humidity by the porometer method, using *Prunus Laurocerasus* and eliminating the action of stomata by applying vaseline to the lower surface of the leaves and then placing their intercellular spaces in communication with the external air by means of incisions. Plotting the results, he found that transpiration varies directly as relative humidity when a correction is made for the fact that the transpiration rate is not zero in saturated air. The fact that transpiration does occur in saturated air is due, as pointed out by SACHS, to the production of heat in the leaf by respiration. The experiments showed that for the transpiration to be entirely checked a humidity of 5 per cent above saturation would be necessary, and hence the temperature of the leaf due to respiration is, under the conditions of the experiments, 0°.8C. above that of the atmosphere.

Using similar methods and materials, DARWIN[8] also studied the effect of diffuse light upon transpiration. The results show so remarkable an amount of variation that it seems dangerous to draw any conclusions other than that light tends to increase the water loss for some unknown reason when its influence upon the action of stomata has been eliminated. This increase averages about 33 per cent.

[6] LIVINGSTON, B. E., and SHREVE, EDITH B., Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. Plant World **19**: 257–309. 1916.

[7] DARWIN, F., On a method of studying transpiration. Proc. Roy. Soc. London B **87**: 269–280. 1914.

[8] ———, The effect of light on the transpiration of leaves. Proc. Roy. Soc. London B **87**: 281–299. 1914.

In a more recent report by the same investigator,[9] the corrections previously indicated for humidity and light are used in experiments designed to show the relation between the rate of transpiration and stomatal aperture, the latter condition being determined by the use of the porometer. There was also applied a further correction for cuticular transpiration. The final results show many irregularities, but are regarded by DARWIN as giving substantial support to his thesis that transpiration is regulated by stomatal aperture. He apparently finds nothing corresponding to the incipient drying of LIVINGSTON and BROWN, or the saturation deficit of RENNER, although it seems possible to the reviewer that some of his many irregularities might require some such explanation.

In striking contrast to this theory there comes an account of a study of water relations of cacti. On account of their peculiar behavior, these plants offer special advantages as well as special problems in the general study of transpiration. Their transpiring power differs from ordinary plants in being greater during the night than during the day. This behavior Mrs. SHREVE[10] has investigated, and has found that there is a regular diurnal march of change in the water-holding capacity of the internal tissues that seems both directly and indirectly responsible for the changes in transpiring power; that is, the transpiring power of the cactus is usually greater at night than during the day because the water-holding capacity of the tissues is greater by day than by night. The variations in water-holding capacity act upon transpiring power indirectly by closing the stomata, which in cacti are usually closed during the day and open at night, and they also act directly by resisting the evaporating power of the air. It seems possible, as the author of this paper points out, that a similar change in the tissues of non-succulents may account for the mid-day drop in their transpiring power.

BRIGGS and SHANTZ[11] have made an extensive and detailed study of transpiration as related to growth and to various climatic factors. The measurements were made at Akron, Colorado, during the seasons of 1914 and 1915 and were for 270 pots of 115 kgm. each of soil, including some 25 different crop plants. Continuous automatic records were obtained for air temperature, solar radiation, wet bulb depression, wind velocity, and evaporation from both shallow and deep tanks. Among the comparisons instituted, one of the most instructive is the correlation between transpiration for the small grains and various physical factors. This is expressed by mean correlation coefficients, some of which are those with evaporation from a shallow pan, 0.87; with wet

[9] DARWIN, F., On the relation between transpiration and stomatal aperture. Phil. Trans. Roy. Soc. London B **207**:413–437. 1916.

[10] SHREVE, EDITH B., An analysis of the causes of variation in the transpiring power of cacti. Physiol. Researches **2**:73–127. 1916.

[11] BRIGGS, L. J., and SHANTZ, H. L., Daily transpiration during the normal growth period and its correlation with the weather. Jour. Agric. Research **7**:155–212. 1916.

bulb depression, 0.88; with temperature, 0.71; with radiation, 0.65; and with wind velocity, 0.22. These serve to emphasize the close relation of transpiration to humidity and evaporation and the comparatively slight influence of wind velocity.

It is also interesting to note that during a 10-day period of maximum transpiration the daily loss of water ranged from 6 to 9 times the dry weight of the crop for millets and corn, from 12 to 16 times for the small grains, and up to 36 to 56 times for alfalfas. During the same 10-day period the annual crop plants lost about one-fourth of the total water transpired during the entire season. The transpiration of the different crop plants per unit area of plant surface shows less variation than the transpiration per unit of dry weight, hence the greater efficiency shown by certain plants in the use of water seems to be due more to a reduction in plant surface than to a reduction of transpiration per unit area of surface. Various other comparisons make this a valuable report for both the botanical and the agricultural investigator.

Another investigation undertaken with a view to economic application of results shows scientific merit of a high order and is comparable in methods and results with that just reviewed. In it KIESSELBACH[12] has limited his research to corn grown under conditions very closely approximating those of crop production. A portion of the experiments was devoted to the development of a satisfactory technique, and errors of former experimenters due to the use of immature plants and small quantities of soil were pointed out. It is impossible to summarize the many data, but it is interesting to note the agreement with the results of BRIGGS and SHANTZ in the very large proportion of total water used by the plant, which is lost during a comparatively short period of maximum transpiration. A rather surprising result is that it was found that corn plants grown for 2 months in a humid greenhouse exhibited no different transpiration rate per unit leaf area when transferred to dry conditions than took place from plants continuously grown under dry conditions. Further it appears that while there were considerable variations in the different varieties in regard to thickness of leaf and epidermis, and also in number of stomata per unit of leaf area, there was no consistent correlation between these structural features and the transpirational rate per unit of dry matter produced. In spite of this it was found that the water requirement of different varieties differed to a marked degree, suggesting that drought-resistant strains may be selected. It also developed as an important result of the investigation that water economy is greatest with neither an excessive nor a deficient soil moisture supply, and further that increasing fertility by the application of fertilizers resulted in still greater water economy.

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[12] KIESSELBACH, T. A., Transpiration as a factor in crop production. Research Bull. no. 7, Neb. Agric. Exp. Sta. pp. 214. *pls.* 4. *figs.* 24. 1916.

The relation of soil moisture to transpiration and to economy in the use of water is also shown by the investigations of YUNCKER,[13] who, using the wax seal method and weighing the sealed pots, has studied the comparative rates of transpiration of young plants of *Zea Mays* growing in soil with 3 different soil moisture contents, all somewhat above the wilting coefficient and showing respectively 25, 45, and 65 per cent of possible saturation. The rate of transpiration and the water requirement for periods up to 1320 hours was least for the driest cultures, which, however, seem to have had no deficiency in water supply, and most for those with the highest soil moisture content. Thus among other things it appears that the experiment demonstrated that the amount of dry matter formed was not at all proportional to transpiration.—GEO. D. FULLER.

Taxonomic notes.—LANGE,[14] in the second part of his studies on the agarics of Denmark, has published his results with *Amanita*, *Lepiota*, and *Coprinus*. The first part, published in 1914, contained a general introduction and an account of *Mycena*. The genus *Amanita* includes 14 Danish species; *Lepiota* 31 species, 1 of which is new; and *Coprinus* 33 species, 3 of which are new. The presentation of each genus is preceded by a full discussion of its characters and an analytical key.

MACBRIDE,[15] in presenting "The true Mertensias of western North America," recognizes 32 species, 4 of which are described as new. GRAY's *Synoptical Flora* (1886) contains 7 species, 2 of which are restricted to the Atlantic states. Since that time, 74 species have been proposed. In a "Revision of the genus *Oreocarya*," 45 species are recognized, 4 of which are described as new. In "Notes on certain Borraginaceae," *Amblynotopsis* is proposed as a new genus (including 4 species transferred from *Krynitzkia*), and new species are also described in *Lappula* (2), *Cryptantha* (2), *Amsinckia*, *Mertensia*, and *Lithospermum*.

MOORE[16] has described 2 new genera: *Capitanopsis* (Labiatae) from Madagascar, and *Megalostylis* (Euphorbiaceae) from Peru or Brazil (upper Amazon region). He also describes 13 new species from Africa.

OKAMURA,[17] in his second contribution to the bryophytic flora of Japan, describes a new liverwort and 29 new species of mosses well distributed generically.

[13] YUNCKER, T. G., A study of the relation of soil moisture to transpiration and photosynthesis in the corn plant. Plant World **20**:151–161. 1916.

[14] LANGE, JAKOB E., Studies in the agarics of Denmark. Part II. Dansk. Bot. Arkiv. **2**:no. 3. pp. 53. *pls. 2*. 1915.

[15] MACBRIDE, J. FRANCIS, Contrib. Gray Herb. no. 48. pp. 58. 1916.

[16] MOORE, SPENCER LEM., Alabastra diversa. XXVI. Jour. Botany **54**:249–257. 1916.

[17] OKAMURA, SHUTAI, Contributiones novae ad floram bryophyton Japonicam. Pars secunda. Jour. Coll. Sci. Tokyo **38**:no. 4. pp. 100. *figs. 42*. 1916.

OVERHOLTS[18] has monographed the Polyporaceae of the central states, including the states extending from Ohio to North Dakota and southward to Kentucky and Kansas. He recognizes 132 species in 7 genera, the species of *Poria* and *Merulius* being omitted because "practically nothing is known of them at present." The large genus is *Polyporus*, with 88 species; following it are *Fomes* with 23 species and *Trametes* with 10 species. The keys and contrasted descriptions should make the identification of species comparatively easy. Perhaps the author is to be congratulated that he did not see fit to propose any new species.

SMITH[19] has described 10 new species and 5 new varieties of algae from the lakes of Wisconsin, and also a new genus (*Gloeocystopsis*), which combines the external morphological characters of *Gleocystis* and *Nephrocytium*.

STEVENS,[20] in a synoptical account of the species of *Meliola* occurring in Porto Rico, recognizes 95 species, and describes 62 of them as new.—J. M. C.

Sulphur nutrition.—Although sulphates have little effect on the soil flora, and cannot function therefore as important fertilizers for all crops, the fact that the sulphur content of most soils is rather low, and that certain classes of plants use considerable quantities of sulphur in metabolism, leads to the possibility of sulphur deficiency becoming in certain cases a limiting factor to crop production. HART and TOTTINGHAM[21] have made some greenhouse studies on the relation of elemental sulphur and various sulphates to the nutrition of certain of the Leguminosae, Cruciferae, and Gramineae, groups differing somewhat in their need of sulphur. They find that sulphates may be beneficial to certain crops, even when functioning only as a source of sulphur.

Calcium sulphate in general gave better results than sodium sulphate. It increased the dry weight produced by red clover 23 per cent. With rape the greatest beneficial influence was noted when the calcium sulphate was used in addition to a complete fertilizer. The increase due to the sulphate in this case was 17 per cent. In both plants the roots were much elongated by the sulphate, so that a much larger volume of soil is laid under contribution to the plant, and its ability to withstand drought is much increased. The sulphate therefore not only meets the special needs of these plants for sulphur but improves the general physiological conditions.

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[18] OVERHOLTS, L. O., The Polyporaceae of the middle-western United States. Wash. Univ. Studies **3**:3–98. *pls. 8.* 1915.

[19] SMITH, GILBERT MORGAN, New and interesting algae from the lakes of Wisconsin. Bull. Torr. Bot. Club **43**:471–483. 1916.

[20] STEVENS, FRANK LINCOLN, The genus *Meliola* in Porto Rico. Ill. Biol. Monographs **2**:1–86. *pls. 5.* 1916.

[21] HART, E. B., and TOTTINGHAM, W. E., Relation of sulphur compounds to plant nutrition. Jour. Agric. Research **5**:233–249. 1915.

The grains, barley and oats, showed little effect on the quantity of straw, but a noticeable increase in seed production occurred on plants grown on the soil used (Miami silt loam).

Elemental sulphur, added as flowers, was usually toxic even in the presence of calcium, probably because of its incomplete oxidation to sulphites. Where bases are deficient, the toxicity may be due to accumulation of sulphuric acid from the complete oxidation of the sulphur.—CHARLES A. SHULL.

British Columbia forests.—Mount Robson, British Columbia, situated at practically the present northern known limit of the continental divide, has been visited by COOPER[22] and found to possess 2 climax forest types, one for each of 2 climatic zones. Up to an altitude of 1000 m. the forest is of the Pacific Coast type, with a dominance of *Thuja plicata*. *Picea Engelmanni* is next in abundance, and is followed by *Abies lasiocarpa*, *Tsuga heterophylla*, and *Pseudotsuga mucronata*. The undergrowth shows such truly mesophytic forms as *Acer glabrum*, *Azaleastrum albiflorum*, *Phegopteris Dryopteris*, *Clintonia uniflora*, *Moneses uniflora*, and *Pyrola uliginosa*.

Above this is a subalpine zone extending up to 2000 m., with a climax forest of *Picea Engelmanni*, *Abies lasiocarpa*, and *Pinus albicaulis*. In the undergrowth *Menziesia ferruginea*, *Cornus canadensis*, and several species of *Pyrola* are conspicuous. The successions upon rock surface, talus, moraine, and shingle flat are noted, those of the two last in most detail. Upon the moraine *Dryas octopetala* and *Arctostaphylos rubra* are followed by shrubby species of *Betula* and *Salix*, leading to the third stage, which is the climax forest. A similar set of stages is found upon the shingle flat, although here, probably because of the lack of any fine soil material, the succession advances much less rapidly than upon the moraine.

While COOPER expresses regret at the few data available for this study, it will be welcomed as giving an insight into the vegetation of an almost unknown region.—GEO. D. FULLER.

Large trees.—A recent contest for two prizes of $100 each, offered through the *Journal of Heredity*,[23] for photographs and data regarding the largest trees in the United States, barring conifers, resulted in photographs of 337 trees. The prize for the largest non-nut-bearing tree was won by a *Platanus occidentalis* near Worthington, Indiana, with a circumference, 5 ft. from the ground, of 42.25 ft., and a height of about 150 ft. The largest nut-bearing tree in the competition was a *Quercus lobata* on the foothills of the Sierra Nevada Mountains, in San Benito County, California, with a circumference of 37.5 ft. and a height of 125 ft. The largest specimens of other species were as follows: *Ulmus americana* at Morgantown, West Virginia, with a circumference of 33

[22] COOPER, W. S., Plant succession in the Mount Robson region, British Columbia. Plant World **19**: 211–238. *figs.* 8. 1916.

[23] Photographs of large trees. Jour. Heredity **6**: 407–429. 1915.

ft.; *Quercus alba* at Atwood, Indiana, 21 ft.; *Juglans nigra* at Hanover Neck, New Jersey, 24 ft.; and *Liriodendron Tulipifera* at Asheville, North Carolina, 34.5 ft.

The report of the results of the contest also contains other interesting data, while the value of such a competition, as pointed out by LAMB,[24] consists not only in promoting interest in the protection of tree individuals and in the conservation and preservation of forests, but also in affording data for the solution of problems of distribution, of growth, and of duration. In this connection he has prepared maps showing the distribution of 6 of the important species represented and the location of the best specimens reported in the contest. It is hoped that public interest in the subject will not cease with the conclusion of the contest.—GEO. D. FULLER.

American forestry.—Recent changes and improvements have made the magazine known as *American Forestry* valuable not only to the forester but also to the botanical teacher or student interested in trees. An excellent feature is that of devoting special attention to one particular tree species in each issue. Well written articles are given dealing with the identification, characteristics, and habits of the trees, and also with the lumber and its uses. During the first half of the current year the following species have been the subjects of special consideration: *Quercus alba*, *Pseudotsuga Douglasii*, *Thuja plicata*, *Betula papyrifera*, *Ulmus americana*, and *Sequoia sempervirens*. The excellence of the illustrations in these articles is worthy of note.

There are also, in addition to the articles of more particular interest to the professional forester, others upon more general but quite as timely topics. Among these we may note as examples a finely illustrated article upon *Cupressus macrocarpa* under the title of "The tree of legend and romance," in the February issue; and several dealing with forests in time of war, showing some of the devastating effects of the present European conflict. A recognition of various phases of forest and country life is seen in regular departments devoted to children, birds, ornamental and shade trees, and to wood preserving, while quite as important are the very extensive lists of current literature. Finally, as an indication of the international scope of its interests is a page of its notes and news items devoted to Canadian forestry and foresters.—GEO. D. FULLER.

A vegetational map of the United States.—SHREVE[25] has compiled a map of the range of the principal types of vegetation in the United States, basing the boundaries of the various subdivisions upon purely vegetational criteria. The primary classes of vegetation are the well recognized ones of desert, grassland, and forest. Of these the first and last have been subdivided, but the data available for the grassland are not regarded as sufficient to afford a basis

[24] LAMB, W. H., Value of the contest. Jour. Heredity **6**:424–429. 1915.

[25] SHREVE, F., A map of the vegetation of the United States. Geog. Rev. **3**:119–125. 1917.

for mapping. On the whole, 18 types of plant communities are recognized, briefly characterized, and plotted. An inspection shows, as its author points out, that the areas have in general a north and south rather than an east and west trend, which tends to show that the major differences in vegetation are here determined more largely by conditions of moisture than of temperature.

While the result is decidedly the best map of the sort yet produced, it is also probable that it would be difficult to find an ecologist who would agree with it in every particular. So much of the disagreement would be differences of opinion as to what should be included within a single vegetational type, that diverse criticism would be neither a gracious nor a practical task, and yet the reviewer cannot refrain from expressing a question as to the fitness of including both the *Pinus ponderosa* and *P. Murrayana* forests of the west and the *P. Strobus*, *Tsuga*, and *Abies balsamea* forests of the east in the "northern mesophytic evergreen forest."—GEO. D. FULLER.

History of forest ecology.—A recent study of the historical development of forest ecology is likely to prove of equal interest to foresters and ecologists. In it BOERKER[26] traces the development of plant ecology from its beginnings to the modern phase characterized chiefly by efforts to measure the various habitat factors. The beginnings of forest ecology are seen in the empirical development of silviculture, dating back to the fifteenth century or even earlier; but the founder of the science is considered to be DUHAMEL DU MONCEAU, about the middle of the eighteenth century. About a century later the work of HARTIG and that of a committee of 5 German foresters, appointed in 1868, consisting of WESSELY, HEYER, EBERMAYER, JUDEICH, and BAUR, gave the science further impetus, and resulted in the organization of a series of forest experiment stations throughout Germany. From this beginning the advancement of the science is traced to the present day, as shown in the work of WAGNER, MEYER, and DUESBERG in Germany, and that of FERNOW and ZON in America. It is notable that not until 1909 were forest experiment stations established in the United States.—GEO. D. FULLER.

Permeability.—It is well established that a great variety of stimuli (light, temperature, salts, anesthetics) modify the permeability of protoplasm. In some cases the stimulus decreases the permeability at low intensity and increases it at high intensity. KOKETSU[27] believes he has demonstrated that electrical stimulation increases the permeability of epithelial cells of *Tradescantia discolor*. Cells thus stimulated are less easily plasmolyzed by ordinary plasmolytic agents than are unstimulated cells. After recovery from the stimulus they show a greater degree to plasmolysis by the same concentration of the

[26] BOERKER, R. H., A historical study of forest ecology; its development in the fields of botany and foresty. Forestry Quarterly **14**:380–432. 1916.

[27] KOKETSU, R., Über den Einfluss der elektrischen Reizung auf die Permeabilität der Pflanzenzellern. Bot. Mag. Tokyo **30**:264–266. 1916.

agent than do the checks. He interprets the first change as due to greater permeability of plasmolytic agents, and the second change as due to loss of solutes during the period of higher permeability. Szücs[28] finds that aluminum salts render cells more difficult to plasmolyze because they harden the protoplasm, although they really decrease its permeability. One must look out for a similar condition with electrical stimuli. The experiments are qualitative but suggest the need of very careful quantitative studies.—WM. CROCKER.

Texas root rot fungus.—DUGGAR[29] has investigated the causal organism of one of the most destructive of the cotton diseases, an organism which seems to be confined largely to Texas, where the average losses have been variously estimated to be $2,000,000 to $3,000,000. In addition to the attacks on cotton, the fungus damages such crops as alfalfa, beans, sweet potatoes, and certain orchard fruits. As illustrating the omnivorous habit of the fungus, DUGGAR enumerates a list of nearly 30 host plants (trees, shrubs, and herbs) already noted as used by the fungus. The chief feature of the disease is the sudden wilting and dying of the affected individuals. The fungus was described by SHEAR as *Ozonium omnivorum*, but DUGGAR concludes that it should be transferred to *Phymatotrichum*. In the revised description of the species the habitat is stated as follows: "Hyphae on living roots of many plants and in the soil; conidial stage on soil in the vicinity of diseased plants."—J. M. C.

Embryo and seedling of Dioscorea.—Miss SMITH[30] has investigated the embryo and seedling of *Dioscorea villosa*, a genus long known through the work of SOLMS-LAUBACH as furnishing evidence of a "second cotyledon," or at least a seedling structure quite different from what had come to be regarded as the monocotyl type. Miss SMITH traced the development of the embryo to the spherical 4-celled proembryo, and then followed the appearance of the organs. She observed no cotyledonary ring, and claims that the single cotyledon originates as a terminal structure. It may be stated that the course of the vascular strands suggests that the leaf called the "first secondary leaf" occupies the position of a "second cotyledon," which would make the growing point of the stem a terminal structure, and both cotyledons lateral. This, however, is a matter of interpretation in connection with material.—J. M. C.

Vitality of moss protonema.—Miss BRISTOL[31] has discovered some remarkable cases of the retention of vitality by the protonema of mosses. In samples of soils obtained from various places for the purpose of ascertaining by means

[28] Rev. in BOT. GAZ. **56**:245. 1913.

[29] DUGGAR, B. M., The Texas root rot fungus and its conidial stage. Ann. Mo. Bot. Gard. **3**:11–23. *figs. 6.* 1916.

[30] SMITH, PEARL M., The development of the embryo and seedling of *Dioscorea villosa*. Bull. Torr. Bot. Club **43**:545–558. *pls. 31–34.* 1916.

[31] BRISTOL, B. MURIEL, On the remarkable retention of vitality of moss protonema. New Phytol. **15**:137–143. *figs. 3.* 1916.

of cultures the algae present in the form of "resting spores," protonema from certain soils began to develop. In these soils the protonema had persisted in the dried condition for 46, 48, and 49 years. A description is given of the appearance of the cells, which seemed to be in vigorous condition. Moss spores contain chlorophyll and are usually short-lived. "Hence the power to produce a resting protonema filament which is able to resume growth, even after half a century, is a great asset to the plant in preventing its extinction through adverse climatic conditions."—J. M. C.

Anatomy of Drimys.—The genus *Drimys* (Magnoliaceae), belonging to the Southern Hemisphere, is very interesting on account of the absence of vessels. JEFFREY and COLE[32] have investigated its wound reactions from material obtained from New Zealand and Java, and also from material at Kew. As a result of injury, the roots develop peculiar tracheary structures, which are regarded as a "reversionary return of vessels" because the markings of the lateral walls resemble those found in the vessels of the Magnoliaceae. They are clearly distinct from ordinary tracheids, but lack the perforations of normal vessels. The authors conclude that these traumatic structures are to be interpreted as a clear indication of the former presence of vessels in *Drimys*.—J. M. C.

A cedar swamp on Long Island.—A swamp on the southern shore of Long Island, New York, about one mile long and half as wide, is, according to TAYLOR,[33] of special interest because (1) it is probably the most northerly grove of *Chamaecyparis thyoides* on the coastal plain of anything like that size; (2) the character of the undergrowth, which includes 77 per cent of species northern in character; and (3) it affords evidence of coastal subsidence in the transition between the swamp and the open salt marsh and in the number of dead and dying trees. This evidence is all the more convincing because of the remoteness of any barrier beach or other possible regulator of exceptional tides, a possible alternative to recent subsidence.—GEO. D. FULLER.

Flora of Isle Royale, Michigan.—COOPER[34] has supplemented his excellent ecological analysis of the vegetation of Isle Royale[35] by a catalogue of its vascular plants. As a list of the mosses of the same island was previously

[32] JEFFREY, EDWARD C., and COLE, RUTH D., Experimental investigations on the genus *Drimys*. Ann. Botany **30**:359–368. *pl.* 7. 1916.

[33] TAYLOR, NORMAN, A white cedar swamp at Merrick, Long Island, and its significance. Mem. N.Y. Bot. Gard. **6**:79–88. 1916.

[34] COOPER, W. S., A catalogue of the flora of Isle Royale, Lake Superior, Michigan. Acad. Sci. Report **16**:109–131. 1914.

[35] ———, The climax forests of Isle Royale. BOT. GAZ. **55**:1–44, 115–140, 189–235. 1913.

published,[36] this catalogue advances the region to the position of having one of the very few well known floras in the state. The present list includes 40 species of pteridophytes and 479 species of spermatophytes. One happy improvement in the present publication is the ecological definition of the habitat, replacing such time-honored but meaningless phrases as "hillsides," "glades," "woods," and "cool dry woods."—GEO. D. FULLER.

Plants of the Florissant lake beds.—These beds in Colorado have been famous for 50 years for their abundance of finely preserved fossil plants and insects. KNOWLTON[37] has now published a review of the plant material on deposit in the U.S. National Museum. Over 100 plants are presented, and among them 18 new species are described, chiefly woody dicotyledons. Two new genera are proposed; *Palaeopotamogeton* (Potamogetonaceae) and *Florissantia* (Solanaceae). The list of types of fossil plants from Florissant in the U.S. National Museum includes the names of 121 species.—J. M. C.

Mushroom fairy rings.—The occurrence of well developed "fairy rings" formed by a large mushroom known as *Tricholoma praemagnum* in the dry grassland of the open mountain parks of Colorado has been described by RAMALEY.[38] They have been observed in various localities, but all between 6000 and 9000 ft. in altitude. The rings vary much in size, the smallest observed being 3.3 m. across, and seen to increase in diameter at a rate of about 1 dm. per year. One of the interesting characteristics of the fungus is its distinctly xerophytic habit.—GEO. D. FULLER.

Aerating system.—HUNTER[39] has studied the structure of various air chambers in plants of *Vicia Faba* and has found spaces of various sorts in the testa of the seed, the cotyledons, the stem, the leaves, and the root. The study adds to our knowledge of the aerating system as developed in seed plants, even if it rather fails to justify the author's conclusion that the system is "elaborately adjusted in order to insure an efficient gaseous exchange for each living cell no matter where its position may be in the plant tissues."—GEO. D. FULLER.

[36] COOPER, W. S., A list of mosses collected upon Isle Royale, Lake Superior. Bryologist **16**:3–8. 1913.

[37] KNOWLTON, F. H., A review of the fossil plants in the U.S. National Museum from the Florissant lake beds at Florissant, Colorado, with descriptions of new species and list of type specimens. Proc. U.S. Nat. Mus. **51**:241–297. *pls. 12–27*. 1916.

[38] RAMALEY, FRANCIS, Mushroom fairy rings of *Tricholoma praemagnum*. Torreya **16**:193–199. 1916.

[39] HUNTER, C., The aerating system of *Vicia Faba*. Ann. Botany **29**:627–634. 1915.